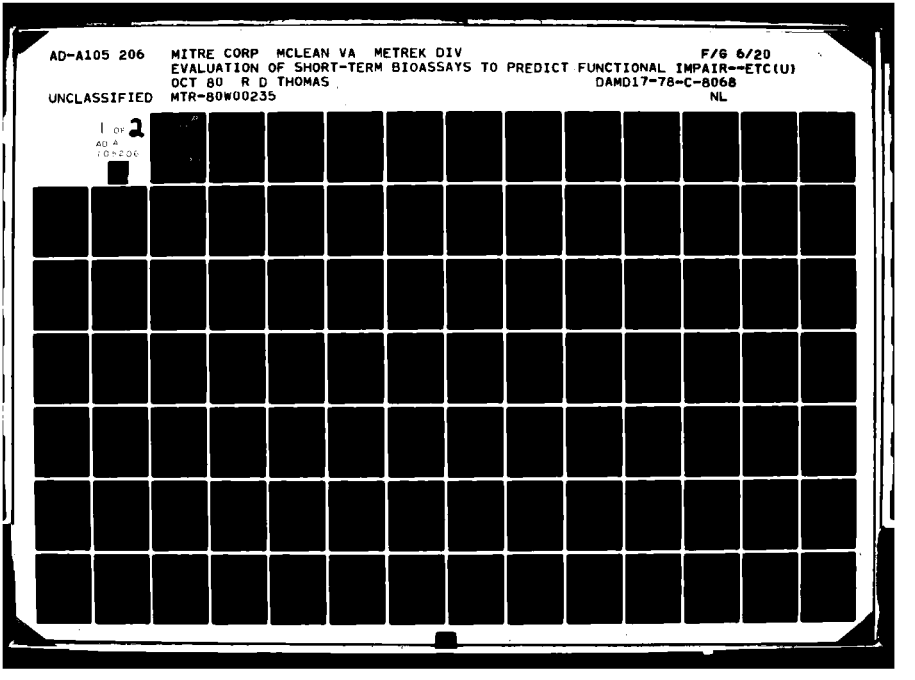
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EVALUATION OF SHORT-TERM BIOASSAYS TO PREDICT FUNCTIONAL IMPAIRMENT

SELECTED SHORT-TERM CARDIOVASCULAR TOXICITY TESTS
Final Report

Richard Thomas

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The MITRE Corporation has been requested by the U.S. Army Medical Bioengineering Research and Development Laboratory to identify and evaluate short-term bioassays which have demonstrated ability to assess and predict impairment of the cardiovascular system resulting from exposure to chemicals. This document reviews the literature on test procedures for determining toxic effects on the heart and other components of the cardiovascular system. The -

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procedures are ranked in descending order of utility and application to a screening program.

The recommended tests include both in vivo and in vitro techniques. The in vivo functional techniques recommended are the monitoring of left ventricular pressure, arterial pressure, aortic flow, cardiac output and electrocardiographic activity. The morphological techniques recommended include gross inspection, light microscopy and limited electron microscopy. The biochemical analyses recommended include serum lactic dehydrogenase (LDH), creatine phosphokinase (CPK) and tissue electrolytes (e.g., magnesium, calcium, sodium and potassium). The in vitro techniques recommended are cultured heart cells and perfused heart preparations. In both the cell cultures and perfused heart preparations, various biochemical (e.g., LDH, CPK) and functional (e.g., beating and electrical activity) parameters may be monitored.

Some experimental procedures currently in the research and development stage are briefly discussed for their future potential as screening tests.

This report is accompanied by a directory entitled Development of Cardiovascular Bioassays in Laboratory Animals: Directory of Institutions/Individuals, which presents the names of organizations and individuals involved in the development and/or utilization of tests applicable to the screening of toxic substances in the cardiovascular system.

EXECUTIVE SUMMARY

The Metrek Division of the MITRE Corporation, under contract to the United States Army Medical Bioengineering Research and Development Laboratory, is reviewing and recommending short-term tests for evaluating and predicting the functional and/or morphological impairment produced by toxic substances using animal test systems. This document presents information on the available tests for the cardiovascular system and recommends those tests which are suitable for use in a screening program.

Cardiovascular damage can be caused by chemicals, disease and various other forms of cardiac stress. A number of cardiotoxic chemical substances have been identified which are found either in the environment, in occupational settings or that have been synthesized by man. The damage these substances cause may be minor (e.g., subtle functional and structural changes) or it may be severe, leading to heart failure. To determine the cardiotoxic activity of a toxicant, the heart and vascular system are examined for lesions. These lesion's may be structural, functional and/or biochemical. In this report, the techniques used to measure cardiovascular damage have been grouped into three categories: morphological, functional, and biochemical.

A variety of testing techniques have been developed to detect cardiovascular damage; however, few of these are well developed or have demonstrated ability to detect damage in short-term screening. Those tests that are sufficiently developed to have potential application in a short-term screening program for cardiotoxicity are described in this report. The information in this report deals only with animal testing. The testing techniques used in humans are included only if they might prove useful in animal testing.

The following criteria have been used to evaluate the cardiovascular system tests described:

- Whether the test is sufficiently developed to be reproducible in a screening program
- Whether the test is sufficiently sensitive to detect early subtle forms of damage and to reflect the extent of damage to the system
- Whether procedures and instrumentation are sufficiently uninvolved to enable technicians with minimium additional training to perform the test

- Whether the test is terminal to the animal used
- The amount of time necessary to complete the test (i.e., days to a few weeks)

After an assessment of the cardiovascular testing techniques was made, none of the techniques sufficiently satisfied the criteria to be immediately useful in a short-term screening program. Nonetheless, a battery of tests are recommended that show the greatest potential utility in a cardiocascular screening program.

The recommended tests include both in vivo and in vitro techniques. The in vivo functional techniques recommended are the monitoring of left ventricular pressure, arterial pressure, aortic flow, cardiac output and electrocardiographic activity. The morphological techniques recommended include gross inspection, light microscopy and limited electron microscopy. The biochemical analyses recommended include serum lactic dehydrogenase (LDH), creatine phosphokinase (CPK) and tissue electrolytes (e.g., magnesium, calcium, sodium and potassium). The in vitro techniques recommended are cultured heart cells and perfused heart preparations. In both the cell cultures and perfused heart preparations, various biochemical (e.g., LDH, CPK) and functional (e.g., beating and electrical activity) parameters may be monitored.

Some experimental procedures currently in the research and development stage are briefly discussed for their future potential as screening tests.

This report is accompanied by a directory entitled Development of Cardiovascular Bioassays in Laboratory Animals: Directory of Institutions/Individuals, which presents the names of organizations and individuals involved in the development and/or utilization of tests applicable to the screening of toxic substances in the cardiovascular system. The information provided for each organization includes specific tests and observations performed; the test systems utilized (e.g., experimental animals or in vitro preparations); the substances administered or conditions established to elicit a toxic response; the use of anesthesia, and the terminal nature of the tasks conducted.

FOREWORD

The authors express their appreciation to Dr. Mary Henry, Project Officer of the U.S. Army Medical Bioengineering Research and Development Laboratory, for the support and guidance that she provided during the course of the project. The expert contributions by David Acosta, Ph.D. and Stephen J. Kopp, Ph.D., who submitted critical reviews of this report in its draft form, is gratefully acknowledged. Leadership and advice by Dr. Paul Clifford and Dr. Barbara Fuller throughout the course of the project have been of great value. The editorial and technical assistance by Ms. Lee Johnson and Ms. Yasuko Anglin, respectively, is sincerely appreciated.

Citations of organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

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TABLE OF CONTENTS

	Page
EXECUTIVE SUMMARY	1
FOREWORD	3
LIST OF TABLES	7
1.0 INTRODUCTION	9
2.0 MEASUREMENTS OF CARDIOVASCULAR DAMAGE	13
2.1 Morphological Techniques	14
2.1.1 Gross Examination and Light Microscopy	14
2.1.2 Electron Microscopy	15
2.1.3 Vascular Structural Changes	17
2.1.4 Primary Heart Cell Cultures	18
2.1.5 Tissue Explants and Perfused Heart Preparations	20
2.1.6 Summary	21
2.2 Functional Testing Techniques	23
2.2.1 Assessment of Cardiac Function	24
2.2.2 Functional Monitoring	25
2.2.3 Electrocardiography	28
2.2.4 Cultured Heart Cells	34
2.2.5 Tissue Explants and Perfused Heart Preparations	35
2.2.6 Summary	38
2.3 Biochemical Techniques	39
2.3.1 Serum Enzymes	40
2.3.2 Electrolyte Levels	43
2.3.3 Other Biochemical Monitoring	46
2.3.4 Cultured Heart Cells	48
2.3.5 Tissue Explants and Perfused Heart Preparations	49
2.3.6 Summary	50
3.0 CONCLUSIONS AND RECOMMENDATIONS	53
3.1 Criteria Used in Evaluating Cardiovascular System Tests	54
3.2 Evaluation of Cardiovascular Tests for Potential Application to a Screening Program	55

TABLE OF CONTENTS

		Page
4.0	REFERENCES	63
APPENDIX A	MORPHOLOGICAL MEASUREMENTS OF MYOCARDIAL DAMAGE	83
APPENDIX B	MEASUREMENTS OF VASCULAR STRUCTURAL DAMAGE	85
APPENDIX C	FUNCTIONAL MEASUREMENTS OF MYOCARDIAL DAMAGE	87
APPENDIX D	BIOCHEMICAL MEASUREMENTS OF MYOCARDIAL DAMAGE	89
APPENDIX E	CULTURED HEART CELLS IN MEASURING MYOCARDIAL DAMAGE	93
APPENDIX F	TISSUE EXPLANTS AND PERFUSED HEART TECHNIQUES IN MEASURING MYOCARDIAL DAMAGE	95

LIST OF TABLES

Table Number		Page
2-1	The Calculation of Functional Parameters from Heart Rate, Mean Blood Pressure and Cardiac Output Values	29
3-1	Evaluation of Cardiovascular Testing Techniques	56
3-2	Recommended Tests for a Batch Screening Program to Detect Cardiovascular Damage	58
A-1	Morphological Measurements of Myocardial Damage	84
B-1	Measurements of Vascular Structural Damage	86
C-1	Functional Measurements of Myocardial Damage	88
D-1	Biochemical Measurements of Myocardial Damage	90
E-1	Cultured Heart Cells in Measuring Myocardial Damage	94
F-1	Tissue Explants in Measuring Myocardial Damage	96
F-2	Perfused Heart Techniques in Measuring Myocardial Damage	97

PAGE

1.0 INTRODUCTION

The Metrek Division of The MITRE Corporation, under contract to the United States Army Medical Bioengineering Research and Development Laboratory, is reviewing and recommending short-term tests for evaluating and predicting the functional and/or morphological impairment produced by toxic substances using animal test systems. Effects in four organ systems--pulmonary, hepatic, renal and cardiovascular--are being considered. This document presents information on the available tests for the cardiovascular system and recommends which tests should be further developed for use in a screening program.

Cardiovascular damage can be caused by chemicals, diseases and various other forms of cardiac stress. Many chemical substances have been identified that induce cardiovascular damage; fortunately few are common substances. Cardiotoxic substances have been found in the environment, in occupational settings and in food. Some commercially produced chemicals such as drugs, pesticides and food additives have been found to be cardiotoxic. The damage these substances cause may be minor (e.g., subtle functional and structural changes) or it may be severe, leading to heart failure. Chemical substances that have the potential for broad exposure, or for which there is the potential for high individual exposure, should be examined for cardiotoxic activity. To determine the level of cardiotoxic activity, the heart and vascular system are examined for agent-induced lesions. These lesions may be structural, functional or biochemical.

The most extensive testing in the cardiovascular system has been in the examination of the heart for functional changes. Efforts have then been made to correlate these functional changes with structural and biochemical alterations. The first section of this report describes the techniques used to detect and determine the extent of structural alterations in the heart. These include the use of gross inspection, and light and electron microscopy in the pathologic description of the heart and of three in vitro systems: cultured heart cells, tissue explants and perfused heart preparations. The second section describes the techniques used to examine the heart for functional changes and damage. These techniques are used to determine heart rates, levels of pressure and flow at various sites in the cardiovascular system, and the electrical conduction patterns and rates in the heart. Techniques used to measure functional parameters in cultured heart cells, tissue explants and perfused heart preparations are also described in this section. The final section describes the biochemical alterations in damaged hearts and in model in vitro systems, and the techniques used to measure these alterations. Specifically described are the use of serum enzyme and tissue electrolyte levels to detect cardiac damage.

The information contained in this report has been compiled from published and unpublished reports, and communications with individuals active in the development of testing techniques for cardiovascular damage. A companion directory of individuals and organizations

involved in cardiovascular testing in animals has been compiled solely from personal communications, so that only the current activities of organizations and researchers would be represented. The information in this report deals only with animal testing. Considerable research has been done concerning human cardiovascular disease, especially in developing human cardiofunctional testing. These testing techniques for humans have only been included in this report when they might prove useful in animal testing.

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2.0 MEASUREMENTS OF CARDIOVASCULAR DAMAGE

Measurements of alterations in structure and function of the cardiovascular system have been used as indices of damage. Attempts have been made to correlate these alterations with biochemical changes. Many techniques for monitoring functional changes have only been used in man, especially the noninvasive techniques. These have not been applied to small animals because of their complexity, and because invasive techniques are more practical and reliable in small animals. Much of the information necessary to evaluate the reliability, sensitivity and capability of individual techniques for predicting and evaluating cardiovascular damage in animals is unavailable. The techniques used both in animals and in humans are in most cases much more developed in humans.

The techniques used to measure cardiovascular damage have been separated into three categories: morphological, functional and biochemical. Some techniques could conceivably be included in more than one category; however, the techniques have been placed in the category that best described the parameters they measure. The in vitro systems (i.e., cultured heart cells, tissue explants and perfused heart preparations) have been described in each category based upon the parameters examined. For example, measurements of enzyme leakage from damaged cultured heart cells is described in the Biochemical Section. The information in each section is augmented by material tabulated in the appendix. The emphasis in this report is on

describing and evaluating the suitability of each technique for potential application to a cardiovascular screening program.

2.1 Morphological Techniques

The most common techniques used to detect damage in the heart have been the gross (macroscopic) and histological (microscopic) examination of the heart. The histological techniques involve the use of light microscopy, transmission electron microscopy (TEM), and to a limited extent, scanning electron microscopy (SEM).

2.1.1 Gross Examination and Light Microscopy

Gross pathological inspection of the heart has important value in a thorough description of cardiac damage. The lesion patterns for many classes of cardiotoxic substances are well known. Many of these lesion patterns in rats have been described by Selye (1961). Changes in the normal size and shape of the heart (e.g., hypertrophy, protruding infarct), the vascular pattern (e.g., arteriosclerosis, calcification), and color (e.g., ischemia, calcification, necrosis), are all important macroscopic features that can be detected by gross inspection.

Most of the early structural features of damage are microscopic and cannot be detected by gross inspection. These features require either light or electron microscopy to be detected. The resolution of light microscopy is restricted by the wave length of the incident light source which limits the examination of heart-cell features to structural components with dimensions greater than 2,000 $\mathring{A}$

(Fawcett, 1969). The necrotic changes in heart cells that can be detected by using light microscopy include: disorganization of cell cytoplasm (e.g., vacuolization, transverse banding); increased amounts of lipofuscin, mineralization, myofibrillar granularity, and degeneration (either pycnosis or enlarged nucleoli); Z-band degeneration, and interstitial edema. Also, leukocytic and histiocytic infiltration and fibroblastic changes can be detected in necrotic tissue, and the extent of necrotic foci can be determined by using light microscopy.

2.1.2 Electron Microscopy

Some early changes in the injured heart cells cannot be detected by using light microscopy. Many investigators have reported dose-related ultrastructural changes in the absence of abnormalities on light microscopy (Rabkin, 1979). The cardiac-cell mitochondria are approximately 2-3 microns long and are easily seen using light microscopy; however, the cristae, which are approximately 100 Å in diameter and about 1 micron long, are difficult to observe. Therefore, the disorganization and loss of cristae during early damage must be detected using electron microscopy. The other kinds of electron microscopic changes observed include: mitochondrial swelling, electron-dense intramitochondrial granules, membranous whorls in the mitochondria or close to the mitochondrial surfaces, severely degenerated mitochondria, nuclear chromatin clumping, sarcoplasmic reticulum dilation, and disruption and myofibrillar degeneration.

The response of the myocardium to cardiotoxic agents varies considerably among animal species (Mettler, Young and Ward, 1977). Furthermore, major differences have been found in ultrastructural changes among species that have the same gross pathologic conditions or are treated with the same substances. Hearse et al. (1976) found rat and mouse hearts to be resistant to anoxia, whereas guinea pig and rabbit hearts were susceptible to the early onset of damage and ultrastructural alterations. Biochemical and physiological differences between species can be identified that may account for some of these differences. For example, those species with especially high serum calcium levels might be more susceptible to damage from catecholamines because these substances increase calcium transmembrane influx.

Many pathological studies of the heart use TEM because: the technique is not difficult to perform, the resolution is much greater than it is with light microscopy, and many facilities doing pathology have an electron microscope. However, electron microscopy has some disadvantages. Even though it is reasonably rapid and simple to perform for a few samples, in a screening program where numerous samples are involved, the sample preparation, handling, and storage becomes difficult and time-consuming. Furthermore, the proper interpretation of early ultrastructural changes may require detailed histopathological analysis and the interpretation may be subject to dispute.

Table A-1 in Appendix A lists many of the substances that have been tested for cardiotoxicity using morphological techniques, and the animal species that were examined.

2.1.3 Vascular Structural Changes

The techniques used in studying changes in vascular structure involve morphological examination of vessels using either light or electron microscopy. This examination of vessels has not been limited to the heart, but includes other organs where the vessels are easily examined microscopically. The vessels that have been examined are found in the following tissues: gastrointestinal mucosa, kidneys, lungs (McKay, Linder and Cruse, 1971); mesentery (Huttner, Jellinek and Kerenyi, 1968); buccal mucosa (Kahn, Johnson and DeGraff, 1971); cremaster muscle (Cotran, 1967); and skin folds (Willms-Kretschmer and Majno, 1969; Stearner and Christian, 1971). In some studies (Cotran, 1967; Stearner and Christian, 1971; Willms-Kretschmer and Majno, 1969), colloidal carbon black or stains (e.g., Evans blue) are injected into the circulation to label the smaller vessels.

These techniques have been used solely for research purposes in studying capillary thrombosis or fibrous tissue proliferation and the narrowing of the vessel lumen following damage. The techniques are not uniform and would require extensive development before they could be considered suitable for screening of chemical substances in a testing program. Table B-1 in Appendix B lists the techniques employed by several investigators in studying vascular damage.

2.1.4 Primary Heart Cell Cultures

The toxicity of cardioactive substances can be investigated by examining primary cultured heart cells for cytotoxic changes. These cultured cells have essentially the same cytologic features as intact tissue cells and exhibit damage in a similar manner.

The heart cell cultures are generally prepared by excising the whole heart or the ventricles from the animal and mincing the tissue with scissors or scalpel blades. The minced tissue pieces are then treated with a proteolytic enzyme (e.g., trypsin) to loosen the tissue matrix so that the cells are free to disperse. In most studies, tissues from embryonic or early postnatal hearts are used because the cells are more easily freed from these tissues than from older tissues, and they exhibit better viability *in vitro*. The dispersed cells are collected and cultured primarily as monolayer sheets, although the heart cells can be studied in various other arrangements in addition to monolayer sheets. For example, the cells can be suspended in culture media, plated on glass or plastic, or attached to some other substrate, such as thin filaments, to produce strands of cells. The cells can also be aggreated to form small spheres (Sperelakis and McLean, 1978).

Cultured heart cells have many of the advantages of other *in vitro* cellular systems in screening chemical substances; they are easier to work with than whole animals and the cytologic alterations are not influenced by systemic effects. These cells are easily

examined using either light- or electron microscopy, and the cytotoxic changes observed are similar to those changes observed in intact cells.

Because cell cultures are relatively easy to prepare and treat, many substances can be screened at one time. Another important advantage is that nearly pure muscle-cell cultures can be produced (Acosta, Wenzel and Wheatley, 1974). Also, other cell types (e.g., enothelial cells) can be easily distinguished morphologically from the muscle cells (Sperelakis et al., 1974).

There are, however, some disadvantages to heart-cell cultures. A major disadvantage of all *in vitro* cellular cultures is the difficulty in extrapolating the experimental results from these single-cell systems to whole animals. The lack of retention of the highly differentiated properties in newly-cultured cells can be a problem. Because young heart tissues are used, the cultured cells are immature; there is a lack of myofibril development and a failure of the sarcoplastic reticulum to organize. Some older cells in culture show morphologic reversion, where there is a loss of myofibril organization and the cells appear cytologically the same as young cells. The cause of this reversion is not clearly known; however, cells do tend to retain their high level of differentiation when the growth of non-muscle cells (e.g., fibroblastic cells) is inhibited either by eliminating a required nutrient (e.g., arginine) from the culture media, or by adding a growth-inhibiting chemical agent to the media (Acosta,

Wenzel and Wheatley, 1974). Because most chemical inhibitors exhibit some toxic effects on muscle cells, care should be exercised to weigh these effects properly when the final results are interpreted.

More developmental work is needed before heart-cell cultures will provide unequivocal results and be generally useful in screening chemical substances for cardiotoxicity. There are no standardized culture procedures for heart cells. Most cultures are done using either rat or chicken hearts, although a few other small animals have also been used (e.g., mice, guinea pigs, rabbits). Table E-1 in Appendix E lists a number of substances that have been examined using cultured heart cells.

2.1.5 Tissue Explants and Perfused Heart Preparations

The entire heart or tissue explants from various sites in the heart can be removed from laboratory animals and maintained in oxygenated perfusion media. The tissue-explant techniques are used principally to examine specialized functions in specific tissues, such as the conduction velocity in the Purkinje Fibers, and are not used to monitor gross or histological changes.

Perfused heart preparations have only been examined to a limited extent for structural changes after chemical treatment. They are not normally used for this purpose. Those that have been examined have shown ischemic changes immediately following their transfer to a perfusion apparatus (Aronson and Serlick, 1976). These changes were probably the result of muscle hypoxia produced during the transfer of

the heart from the animal to a perfusion apparatus without a continuous supply of oxygen. These changes would limit the usefulness of examining the muscle for structural changes. Most studies using perfused heart preparations have been limited to examining the hearts for metabolic changes, although a few investigators have also examined the heart preparations for structural changes (Su and Chen, 1979; Taam et al., 1979). A more detailed discussion of tissue explant and perfused heart techniques will be presented in the next two sections. Studies using tissue explants are summarized in Table F-1 and perfused heart techniques are summarized in Table F-2 in Appendix F.

2.1.6 Summary

The structural changes in the heart for many cardiotoxic substances are well documented in the literature. The techniques used in gross observation, and light- and electron microscopy, are well developed. Gross observation is limited to the description of relatively severe changes in the heart, although it is an essential part of any pathologic examination procedure. Light microscopy can detect necrotic changes in tissues, such as edema; disorganization of vesicular structures; and many cellular changes, such as vacuolization of cytoplasm, myofibril degeneration and other marked changes in cellular morphology. However, light microscopy is limited in the level of cellular structures that can be resolved. Lesions on the order of 2,000 $\mathring{A}$ or less are beyond the limits of light microscopy

and must be resolved using electron microscopy. This technique is the most useful method currently available for detecting early structural changes. Many early changes (e.g., mitochondrial changes) can only be observed using electron microscopy. This technique is reasonably rapid and simple to perform when only a few samples are involved; however, in a screening program with numerous samples, electron microscopy would be arduous to perform. Light microscopy is more simple to perform than electron microscopy, even though it would still be time-consuming when many samples are involved. However, it may be useful for interpretating other experimental results in a screening program. Even though the histopathology of many substances is clearly described in the literature, the analysis of changes induced by substances of unknown toxicity would be difficult in a short-term screening program and the interpretation of the results would be subject to dispute.

The observation of morphological changes in primary heart-cell cultures would be a worthwhile aspect of the use of this in vitro system in a screening program. Morphological observation of tissue explant and perfused heart preparations would be limited to the confirmation of functional and biochemical changes and would not be useful as an isolated monitoring procedure when these techniques are used.

2.2 Functional Testing Techniques

The heart is a dynamic organ that adjusts to variable requirements for cardiac output (i.e., oxygen demand) by normal homeostatic mechanisms. These mechanisms can vary output as much as five to sixfold, based on normal demands. There are some mechanisms that provide reserve capacity to the heart so that it can cope with various forms of stress, including insult and damage. The mechanisms of reserve capacity include the following: increased heart rate, increased stroke volume, increased oxygen extraction, redistribution of blood, anaerobic metabolism, cardiac dilatation and cardiac hypertrophy (Hurst et al., 1978). These mechanisms are interrelated and have varied influence on each other; a change in one may induce a change in another. They cannot be distinctly separated because of their overlapping nature; thus monitoring cardiac function requires examining and correlating multiple parameters.

Even in severe cardiac damage, the heart may be able to compensate sufficiently to maintain many normal functional indices. For example, investigators (Gould, Lipscomb and Hamilton, 1974) have shown that the resting blood-flow rate remained normal in a coronary artery until the coronary stenosis exceeded 85 percent. One of the most serious conditions in the general population is progressive silent coronary disease that goes undetected, mainly because of the heart's ability to compensate for damage and continue to function in what seems to be normal functional ranges. Because the heart has

considerable reserve capacity and resilience, functional monitoring can have only limited application in a screening program. Screening program techniques should detect early changes and damage in the heart to be useful; however, most functional monitoring has the limitation of being insensitive to early cardiac damage. The damage must be severe and advanced before functional parameters are significantly altered.

2.2.1 Assessment of Cardiac Function

When assessing cardiac function, many physiological parameters can be monitored. The most important of these include heart rate, arterial blood pressure, left-ventricular pressure, aortic pressure and aortic flow. These few parameters can provide an indication of overall cardiac function. One reason for monitoring left ventricular pressure is to determine the left ventricular end-diastolic pressure (LVEDP). Based on the Frank-Starling Law, the LVEDP is the ventricular preload; the aortic pressure is the ventricular afterload. The Frank-Starling Law states that the greater the heart is filled during diastole, the greater will be the quantity of blood pumped into the aorta. Starling was able to show that the presystolic fiber length correlated with cardiac responsiveness to changes in venous input and cardiac output. Since end-diastolic fiber length is difficult to determine in a functioning heart, left ventricular end-diastolic pressure is measured because both are closely related. Nonetheless, it should be noted that small changes in filling pressure are

associated with significant increases in cardiac work as fiber length changes. Any damage to or loss of muscle fiber will decrease the strength of ventricular contraction.

The afterload is the peripheral vascular resistance against which the ventricles contract. The major factors producing afterload for the left ventricle are aortic impedance, peripheral vascular resistance, and the mass and viscosity of the blood. The afterload influences the systolic emptying of the ventricles and indirectly influences the character of the next ventricular beat.

2.2.2 Functional Monitoring

Most monitoring of cardiovascular function in laboratory animals is accomplished using invasive procedures. Much of the early monitoring of cardiovascular function, particularly during the elucidation of cardiovascular physiology, was done on laboratory animals under general anesthesia with the chest open, and the necessary instrumentation was applied directly to the heart or vessels (Vatner, 1978). For instance, the cardiac contractile force and heart rate can be measured with a strain-gauge arch, sutured directly to the left ventricle (Liu and Williams, 1971; Eikenburg and Stickney, 1979); and the left ventricular pressure can be measured by puncturing the ventricle with a needle attached directly to a pressure transducer (Dowell, Cutilletta and Sodt, 1975). These procedures have the disadvantage of potentially altering the cardiovascular dynamics being monitored. The recently developed techniques using

catheters employing electrical transducers to monitor pressure and flow have increased the ease of monitoring cardiac function. Most of the pressure and flow monitors are maneuvered fluoroscopically into position, or they are surgically implanted and provide an opportunity for continuous monitoring. The invasive monitoring techniques are not uniform; indeed, they usually vary from one researcher to another. Also, a researcher may use a combination of open-chest procedures, catheters, manometers and specialized mechanical gauges, and a combination of both invasive and noninvasive techniques. When catheters are used, the positioning in the heart or vasculature will depend upon the researcher's judgement and preferences, the experimental design and the species of animal used. The surgical procedures used for implantation will also vary from one researcher to another (Eikenburg and Stickney, 1979; Scott and Cowley, 1969; Rowe et al., 1963; Pasyk et al., 1971; Regan et al., 1969; Brobmann, et al., 1970; Cramlet, Erickson and Gorman, 1975).

Many functional indices are calculated from parameters that are measured. These include cardiac output, cardiac index, mean cardiac power, cardiac work, stroke volume and total peripheral resistance. Cardiac output--the volume of blood per minute the heart pumps--is a common parameter determined to assess cardiac function, and provides a general indication of overall function. Cardiac output has been determined by the direct Fick techniques (Walsh, Tsuchiya and Frohlick, 1976; Rowe, et al., 1963; Scott and Cowley, 1969; Adams and

Cole, 1975), using either dye dilution or isotope dilution (Liu, 1976; Pasyk, et al., 1971; Rowe et al., 1963; Taylor and Drew, 1975; Morvai and Ungvary, 1979); or by indirect calculation from other functional parameters (Dowell, Cutilletta and Sodt, 1975; Cramlet, Erickson and Gorman, 1975; Brobmann, et al., 1970). The Fick principle states that cardiac output is equal to oxygen consumption, divided by arterial-blood oxygen content, minus venous blood oxygen content (Hurst et al., 1978). Therefore, using the Fick technique requires accurate determination of oxygen consumption for the laboratory animal and accurate determinations of arterial and venous blood oxygen content. Accurate determination of oxygen consumption in laboratory animals can be particularly difficult and requires the use of an enclosed or regulated breathing system.

The dilution techniques depend upon determining the concentration of a dye, isotope or other substance at various time intervals downsteam from the point of injection. When the indicator-dilution curve is plotted to give the rate of dilution, the mean volume rate of the blood flow can be calculated. The mean volume rate of flow is the same as the cardiac output (i.e., the volume of blood pumped by the heart each minute). Once the cardiac output has been determined, the cardiac index is calculated by dividing the cardiac output by the body surface area. The mean cardiac index for normal rhesus macaques (2.5-5.6 kg - female) has been found to be 4.58 ± 0.83 liters/minute for each square meter of body surface area (L/min m^2). The range

for the cardiac index was 3.0-6.3 L/min m^2 (Liu, 1977), which shows the variability of normal baseline values, depending on the animal used and other experimental variables. The formulas for calculating functional indices (Liu and Williams, 1971) from the values for heart rate, mean blood pressure and cardiac output are shown in Table 2-1.

Normal baseline values have not been determined for the functional parameters monitored in most laboratory animals, so abnormal values have not been investigated for known cardiotoxic agents. Baseline values could conceivably be established for specific strains of animals used in a screening program, and in most studies they are determined for control animals. Measurements of cardiovascular function which have been performed in animals using both invasive and noninvasive techniques are outlined in Table C-1 in Appendix C.

2.2.3 Electrocardiography

The transmission of a depolarization wave through the heart causes electrical currents to spread into tissues surrounding the heart. This current is conducted to the surface of the skin, where it can be monitored. The recording of these electrical potentials generated by the heart from the surface of the skin is called electrocardiography (ECG).

The normal electrocardiogram is composed of a P wave, a QRS complex and a T wave. Most mammalian electrocardiograms have these same basic patterns since most mammalian hearts have similar structure. However, there are important variations from species to species in

TABLE 2-1

THE CALCULATION OF FUNCTIONAL PARAMETERS FROM HEART RATE, MEAN BLOOD PRESSURE AND CARDIAC OUTPUT VALUES

PARAMETER		FORMULA
Mean cardiac power (Joule/sec)	=	mean blood pressure (mm Hg) x cardiac output (liter/min) x 0.0022[c]
Stroke Volume (ml/beat)	=	$\frac{\text{cardiac output (ml/min)}}{\text{heart rate (beats/min)}}$
Cardiac Work (Joule/beat)	=	mean blood pressure (mm Hg) x stroke volume (ml/beat) x 1333[b]
Total peripheral resistance[a] (*dyne · sec/cm*^{5})	=	$\frac{\text{mean blood pressure (mm Hg)}}{\text{cardiac output (liter/min)}} \times 1333^{b} \times \frac{60}{1000}^{d}$

[a] The vascular resistance to blood flow from the heart is estimated by analogy to Ohm's law:

$$\text{Resistance} = \frac{\text{volts (pressure)}}{\text{amps (flow)}}$$

Therefore: Total peripheral resistance = $\frac{\text{pressure (mean blood pressure)}}{\text{flow (cardiac output)}}$

[b] 1 mm Hg (pressure) = 1,333 dyne/cm^2

[c] 1 Joule/sec = 0.0022 $\frac{\text{mm Hg} \cdot \ell}{\text{min}} = \frac{(1{,}333 \text{ dyne/cm}^2)(1000 \text{ cm}^3)}{(60 \text{ sec})}$

[d] Conversion of 1 liter to 1000 cm^3 and 1 minute to 60 seconds

Adapted From: Liu and Williams, 1971

the patterns observed. The P wave is caused by the electrical currents from the atria as they depolarize prior to contraction. The QRS complex is actually composed of three separate waves (i.e., the Q, R and S waves) caused by currents from the ventricles as they depolarize prior to contraction. The T wave is caused by the repolarization of the ventricle. The atria also repolarize to form a T wave; however, this occurs at approximately the same time as the QRS complex and is almost always totally obscured (Hurst, 1978; Rabkin et al., 1979; Balazs, 1973).

The intensity of the waves (i.e., the amount of voltage recorded), and the distance between wave patterns, will depend on the animal species used, the distance at which the electrodes are placed from the heart, and the orientation of the electrodes relative to the heart. The greater the distance the electrodes are placed from the heart, the lower the current will be on the skin surface. The standard placement of the electrodes is one on each forelimb and one on the left hind limb. This is Einthoven's triangle, superimposed on the chest and limbs of the animal being monitored. It does not matter much which ECG lead is monitored in a study screening for damage because all the leads give similar wave patterns, although amplitudes vary according to which lead is monitored. When damage is detected, one lead may be better for monitoring a specific area of damage than another. In most studies, only Lead II is used to record ECG, recording the potential difference between the electrodes on the right

forelimb and the left hind limb. The left hind limb has been selected (by Einthoven) as the positive electrode providing for a positive deflection of the QRS complex (Hurst, 1978).

Any pharmacologic or toxic effect to the heart that alters the sequence and/or the rate of depolarization and repolarization will alter the ECG. Pharmacologic effects will be reversible, while toxic effects where damage is produced will not, since the damage has permanently altered the sequence of depolarization and repolarization in the myocardium. For example, if the ventricular muscle is sufficiently damaged (e.g., myocardial infarction, Digitalis intoxication to disrupt the normal depolarization sequence in the ventricles then the QRS complex will be altered. Depending upon the type of damage and the species of laboratory animal used, many different abnormalities can be observed, the most common being unusually prominent waves, prolonged waves, or inverted waves and decreased amplitude.

Electrocardiography is easy to perform and does not require invasive techniques. The most common procedures involve the use of needle electrodes inserted in the skin after the animal has been lightly anesthetized (e.g., Nembutal). The animals can also be trained to allow use of the electrodes without anesthesia, or if repeated or continuous monitoring is required, the electrodes can be surgically implanted.

Because the ECG is easy to perform, many animals can be monitored in a short period of time (i.e., a few hours). Furthermore, a single animal can be monitored many times throughout a program since the procedure causes minimal damage to the animal, depending upon the techniques used to place the electrodes. ECG is a common monitoring technique that has been used in most laboratory animal species, most commonly the rat, dog, rabbit, and monkey; therefore, normal ECG patterns are well known for these animal species. Accordingly, important intraspecies variations have been observed.

The most severe disadvantage of ECG techniques is that they seem to be insensitive to early damage. The damage must be moderate to severe to disrupt the normal current sequences, except possibly when changes occur in the specialized conduction tissue (e.g., His bundle), which seems to be more sensitive to some toxicants than the myocardium, and may exhibit more immediate ECG changes in the form of prolonged conduction intervals. For example, Kopp and Hawley (1978) showed progressive increases in PR intervals in rats exposed for 71 days to low levels of cadmium in drinking water. The PR interval measures conducted time through the atrio-ventricular conduction tissue. ECG conduction intervals, reflecting the sensitivity of the specialized conduction tissue to toxicants, may prove to be more sensitive in detecting subtle toxic changes than the traditional measurements of wave amplitude. The conduction interval measurements are most reliable when repeated measurements are made in the same

experimental animal. Nonetheless, in traditional ECG studies, the damaged area in the myocardium must be large since small current changes are difficult to monitor and discriminate from artifacts of the technique. Such artifacts can be produced by not having the electrodes placed at exactly the same position on each animal, not having each of the electrodes attached properly, or by animal movement during recording. These difficulties can vary the configuration of the QRS complex or the T wave and make quantitation of ECG's between animals difficult (Page et al., 1979).

Finally, in the same species, the size of the animal will also change the ECG because as the distance from the heart increases, the potential decreases. Furthermore, the equipment used for monitoring is not uniform, and small laboratory animals with rapid heart rates require more sophisticated, rapid-recording equipment than do larger animals. Vanarsdel (1979) is currently investigating the possibility of monitoring experimental animals, using the standard limb-leads and an additional forelimb-prescapular lead to give two planes of Einthoven triangles and measure three dimensional cardiac electrical activity. The three-dimensional ECG data require computer analysis. This modified technique or some other future technique, such as monitoring His bundle conduction tissue, may prove more sensitive than current techniques and be capable of detecting early damage to the heart.

2.2.4 Cultured Heart Cells

Heart cells begin beating soon after they are cultured. Therefore, the cells' beating activity and electrical properties can be examined for cytotoxic response when the cultures are treated with chemical substances. For example, treating myocardial cells with ouabain leads to arrhythmias, with eventual cessation of the beating activity (Goshima, 1975).

Heart cells can continue beating in culture for periods as long as ten to twelve weeks (Harary, Fujimoto, and Kuramitsu, 1964; Acosta, Wenzel and Wheatley, 1974). Individual myocardial cells beat at very different rates. When cells come into contact with each other, their rate of beating becomes synchronous. Consequently, when aggregates are formed, they begin beating as a single mass. The electrical properties of the single cells, monolayers or more complex aggregates, such as spheres, can be measured using microelectrodes.

The beating and electrical properties of the heart cells are monitored using photoelectric techniques and microelectrodes, respectively. One difficulty is that the cells can become electromechanically uncoupled and do not contract even though they still have normal firing action. For this reason and because of the variations encountered in beating rates among cells, monitoring beating activity and electrical activity to detect toxic effects is not as reliable as some other cellular parameters (i.e., enzyme leakage). Nevertheless, there have been some recent improvements in monitoring

beating and electrical activity (Wenzel and Kleoppel, 1978; Auclair and Vernimmen, 1980; Vernimmen and Auclair, 1980). The factors that influence beating and electrical activity in the myocardial cells are not well understood.

2.2.5 Tissue Explants and Perfused Heart Preparations

The perfused heart and explanted tissues from the heart will beat spontaneously when maintained with oxygenated perfusion media. The functional properties can then be examined. Some of the tissue explants that have been examined include: papillary muscle, Purkinje fibers (Bigger and Jaffe, 1971; Friedman et al., 1973; Wit, Steiner and Damato, 1970; Freer et al., 1976), His bundle fibers (Lazzara et al., 1976) and aorta segments (Wohl, Hausler and Roth, 1968b; Lundy, 1978; Bult and Bonta, 1976; Orter, Miya and Bousquet, 1975).

The tissue explant techniques are valuable for examining specialized function in specific tissues, such as the conduction velocity in the Purkinje fibers, although the metabolism found in tissue explants might be quite different from that found in intact tissue in a functioning organ. Some of the biochemical changes observed in tissue explants are probably the result of tissue damage inflicted during the surgical explant procedures; thus alterations in functional parameters may also be technique artifacts and may result from effects of toxic substance exposure. Nevertheless, the functional changes monitored in tissue explants are difficult to interpret

without a better understanding of the mechanisms involved, and without further development they would not be useful in a screening program.

Many investigators have monitored the perfused heart for functional parameters such as flow rate, beating activity, contraction force and electrical activity (see Table F-2, Appendix F). In most studies, the flow rates are regulated using a stopcock and the pressure is monitored using a pressure transducer. The contraction force (systolic tension) is normally measured using an apical ligature connected to a force transducer.

Electrocardiographic recordings are made by positioning electrodes on the heart surface for optimal ECG amplitude in a lead II - type configuration. In perfused hearts, His bundle electrograms (HBE) are also made where the inferior vena cava is trimmed near the right atrium and an electrode is positioned in the intra-atrial septal region and adjusted to maximize amplitude. The reference electrode is suspended in the electrolyte solution in the heart chamber (Kopp et al., 1978). HBE measures intra-atrial conductivity (P-A interval) and conduction times through the A-V node (A-H interval) and the His-Purkinje system (H-V interval). Damage or alterations in conduction in these systems can be detected in the HBE.

Aronson and Serlick (1976) examined perfused rat heart preparations to determine the effects of perfusion on the physiological and biochemical state of the isolated preparations. This was done to determine if perfused heart preparations might provide a reliable in

vitro system for screening chemical agents for cardiotoxicity. They found that the isolated perfused heart remained relatively stable biochemically over the period of study (4 hours). The metabolite concentrations and phosphorylase activity showed only minor changes with the exception of glycogen, which decreased significantly after 3 hours of perfusion. During the first hour of perfusion there was a 50 percent decrease in creatine phosphate which continued to decrease slightly throughout the remainder of the study. The heart rate decreased slowly to 86 percent of the initial rate after 4 hours. Significant changes in coronary flow were observed after the first 2 hours of perfusion and the isometric systolic tension (contraction force) decreased significantly throughout the study to approximately 35 percent of the initial value.

Properly prepared perfusate for the heart preparations is essential for maintaining stable viable preparations. For example, Kopp (1980) has found that the use of bicarbonate and phosphate buffers may cause loss of calcium from the perfusate as calcium precipitates, which leads to a loss of functional stability in the preparation. When a Tris-HCl buffer is used (Kopp et al., 1978; Kopp, 1980), preparation functional stability is maintained for more than three hours (after three hours, systolic tension is 86 percent of control).

Perfused heart preparations, although involved, may provide a useful system for screening substances for cardiotoxic potential. The studies using perfused heart techniques are summarized in Table E-2 in Appendix E.

2.2.6 Summary

The techniques necessary for monitoring functional parameters are complex, many requiring surgical intervention, and are varied, depending on the skill and preferences of the researcher. Baseline values for normal laboratory animals are generally unavailable. Baseline values could conceivably be established for specific strains of animals used in a screening program, and in most studies they are determined for control animals. However, because of the heart's considerable reserve capacity and resilience, temporary abnormal values following exposure to a chemical agent cannot be ascribed solely to damage, but may be the result of pharmacologic action. Early damage to the heart usually does not alter functional parameters. Therefore, the measurement of functional parameters as an indication of myocardial damage may have limited application in a short-term chemical screening program except where the detection and monitoring of severe damage is necessary.

Electrocardiographic techniques would have only limited application in a short-term screening program for cardiotoxicity. Since ECG is insensitive to early cardiac damage, its main application would be in detecting severe degenerative changes that would be expressed in the heart in a relatively short period of time (i.e., hours to days). Nevertheless, ECG is useful in monitoring for pharmacologic effects such as increased rate and force of contradiction. Many toxic substances produce pharmacologic effects which may

need to be monitored during a screening program. Vanarsdel (1979) is currently investigating the possibility of monitoring experimental animals by measuring three dimensional cardiac electrical activity. The three dimensional ECG data requires computer analysis. This modified technique or some other future technique such as monitoring solely His bundle conduction tissue may prove more sensitive than current techniques and be capable of detecting early damage to the heart. The disadvantage of the more elaborate ECG techniques is that they require greater data processing capabilities and this would tend to limit their usefulness in a screening program.

The beating and electrical activity of cultured heart cells exposed to cardiotoxic substances can be monitored as indicators of functional toxic effects. However, these parameters are not as reliable in the detection of toxic effects as the monitoring of other cellular parameters, such as enzyme leakage from the cultured heart cells.

The perfused heart preparations are stable systems for functional monitoring and can provide unique opportunities for studying metabolic alterations affecting functional parameters; therefore, most past studies focused on biochemical alterations in metabolism, which affect contraction force or function viability.

2.3 Biochemical Techniques

Damage in the heart increases some serum enzyme levels and is associated with substantial increases in tissue electrolyte levels in

the affected tissue. Therefore, increases in serum enzyme levels and tissue electrolyte levels can provide an indication of cardiotoxicity. The effectiveness of using biochemical methods to monitor for damage in the heart is dependent upon the specificity of the substance monitored (e.g., lactic dehydrogenase for the heart), the intensity of the biochemical alterations, and the ability to assay for the substance in body fluids or tissues.

2.3.1 Serum Enzymes

When cells are damaged, enzymes from the cell leak through the cell membranes into the blood stream, thereby increasing serum enzyme concentrations. Heart muscle tissue has high levels of creatine phosphokinase and intermediate levels of lactic dehydrogenase (Coodley, 1970), both of which leak into the blood stream when the myocardium is damaged. These two enzymes are currently the only ones that are monitored in animal studies for an indication of cardiac damage.

Lactic dehydrogenase (LDH) is widespread in the body and is contained in a number of tissues in the body besides the heart muscle. Disease and damage to organs and tissues other than the heart can cause elevations in serum LDH. Pulmonary, hepatic, renal and muscle damage and disease, anemia and other blood disorders cause increases in serum LDH levels (Coodley, 1970). This lack of specificity is a problem in monitoring LDH for an indication of damage solely in the heart. LDH in serum can be separated into five different components

by electrophoresis. Each fraction is called an isoenzyme. The isoenzyme LDH_1 has been found to be specific for the damaged myocardium and shows promise in cardiac monitoring, although only limited monitoring of this isoenzyme has been made in animals (Tacker, Van Vleet and Geddes, 1979). It is released primarily by the heart; however, damage to red blood cells and renal cortical injury also increase serum LDH_1 levels. Either LDH or LDH_1 has been monitored in dogs (Tacker, Van Vleet and Geddes, 1979), rabbits (Olson et al., 1974) and rats (Olson and Capen, 1978) during cardiotoxicity studies. However, neither LDH nor LDH_1 have been monitored sufficiently in laboratory animals to be immediately useful in a screening program.

Serum LDH activity in quantified spectrophotometrically by following the reduction of the coenzyme nicotinamide adenine denucleotide phosphate (NAD) as lactate is oxidized to pyruvate (Coodley, 1970). LDH_1 is relatively easy to quantify because it is more "heat-stable" than the other four isoenzymes and resists denaturation at 65°C where the activity of the others is destroyed. For this reason it is not necessary to separate the isoenzymes by electrophorsis to quantify LDH_1.

Creatine phosphokinase (CPK) is found in high concentrations in the heart muscle and in skeletal muscle. It is a specific enzyme for muscle tissue (Coodley, 1970). Monitoring serum CPK can provide direct indication of damage to the myocardium; however, skeletal

muscle should also be monitored concurrently for degenerative changes since trauma to the muscle or muscle diseases in skeletal muscle can cause increases in serum CPK. Also, thyroid and some brain diseases can cause increases in CPK levels.

Serum CPK determinations involve the formation of creatine and adenosine triphosphate (ATP). Glucose-6-phosphate is then formed from the reaction of ATP with glucose hexokinase, which reduces nicotinamide adenine denucleotide phosphate (NADP). The reduction of NADP is followed spectrophotometrically at 340μm . Another similar procedure is available for CPK determination, where the oxidation of the coenzyme nicotinamide adenine dinucleotide (NADH) is followed (Coodley, 1970).

The ratio of the CPK isoenzymes MM and MB has been primarily monitored in humans, although in a few studies it has been monitored in dogs (Tacker, Van Vleet, and Geddes, 1979). As with LDH, there has been insufficient monitoring in laboratory animals of CPK or the isoenzymes of CPK to be immediately useful in a screening program. When laboratory animals were treated with adriamycin, increased levels of both LDH and CPK were measured (Olson and Capen, 1978). During chronic feeding studies, significant elevations were observed immediately following the earliest detectable ultrastructural changes (i.e., sarcotubular dilatation); however, other investigators (Tacker, Van Vleet and Geddes, 1979) have found serum enzyme changes insensitive to early mild cardiac damage. With additional

development, monitoring of serum enzymes may prove to be a sensitive method for detecting myocardial damage. The first step in determining their sensitivity for detecting damage, however, is to establish normal baseline values for laboratory animals.

2.3.2 Electrolyte Levels

The monitoring of tissue electrolyte levels has been used to detect damage in the myocardium. In a number of studies, investigators have detected significant alterations in tissue electrolyte levels in damaged tissue; for example, in the ischemic and damaged heart muscle, increased levels of sodium and calcium and decreased levels of potassium and magnesium have been detected (Shen and Jennings, 1972). In studies of myocardial necrosis induced with isoproterenol (Lehr et al., 1966; Fleckenstein et al., 1973; Bloom and Davis, 1972), adriamycin (Olson and Capen, 1978; Olson et al., 1974) and vascular occlusion (Lehr and Chau, 1973), increases were observed in the myocardial tissue concentrations of sodium and calcium, and decreases were observed in potassium and magnesium. Tissue electrolyte levels are determined by either flame photometry or atomic absorption spectrometry.

Many investigators (Ito and Chidsey, 1972; Bloom and Davis, 1972; Shen and Jennings, 1972; Fleckenstein et al., 1973) have reported increased levels of calcium in the myocardium following cellular damage. The administration of beta-sympathomimetic catecholamines increases the force of the beating heart by increasing calcium

transmembrane influx and can lead to calcium loading in the heart cells.

Excessive increases in calcium can damage the myocardium, besides leading to contractile failure by depleting high-energy phosphate stores, i.e., adenosine triphosphate (ATP) and creatine phosphate (Rona, et al., 1959).

The tissue electrolytes normally monitored for cardiac damage are calcium, sodium, potassium and magnesium. These have been monitored in rats, guinea pigs, rabbits and dogs. Calcium is the electrolyte most commonly monitored. The difficulty in using calcium levels as an indicator of myocardial damage is that calcium levels in the myocardium will not vary much as long as there is sufficient ATP available for calcium extrusion from the myocardial fibers. When ATP levels do fall too low, then major increases in intracellular calcium concentrations are observed (Fleckenstein, et al., 1974). Determination of calcium levels in the myocardium can provide an indication of damage; however, baseline information concerning normal tissue calcium concentrations are unavailable and the factors controlling intracellular calcium levels are not well understood.

The ischemic and damaged heart is characterized not only by increased levels of calcium but by increased levels of sodium and tissue water and decreased levels of potassium and magnesium. Investigators (Olson and Capen, 1978; Olson et al., 1974) have shown marked elevations of sodium, calcium and tissue water in the

ventricular myocardium with the accompanying morphologic evidence of tissue damage (e.g., myocyte vacuolization, edema, fibrosis) in animals administered adriamycin. There were also major decreases in the levels of potassium and magnesium. The measurement of elevated tissue electrolyte levels in the myocardium as an indication of damage may prove useful in the evaluation of potential cardiotoxic substances in acute studies when more baseline information has been obtained; however, procedures are insufficiently developed to be useful in a chemical screening program. Lehr has recently completed studies (1980) which show that monitoring tissue electrolyte levels in heart tissue is a very sensitive technique for detecting early subtle damage, and is more sensitive than serum enzyme levels in monitoring cardiac damage. This seems to be especially true of tissue magnesium loss which seems to be a very sensitive indicator of early myocardial damage (Lehr et al., 1975; Lehr, 1980; Lukacsko, 1979).

A major difficulty in monitoring tissue electrolyte levels is that the myocardial electrolyte homeostatic mechanisms interfere with electrolyte determinations for periods longer than a few days. Some chronic studies (Lehr and Chau, 1973; Shen and Jennings, 1972) have reported no significant changes in electrolyte levels in damaged animals. Care should be exercised in the interpretation of tissue electrolyte changes since some changes may not be biologically significant. Consequently, studies of tissue electrolyte changes should

be corroborated with other studies examining structural or functional parameters. Serum electrolyte levels are not useful in detecting cardiac damage (Olson et al., 1974).

Monitoring serum enzyme and tissue electrolyte levels could prove useful in screening potentially cardiotoxic agents; however, developmental work is needed. Especially necessary at this time is baseline information concerning normal levels of these substances in experimental animals and better correlation with abnormal structural or functional changes observed during damage. Table C-1 in Appendix C lists the biochemical measurements made in testing cardiotoxic agents.

2.3.3 Other Biochemical Monitoring

Modification of the processes leading to the synthesis of DNA, RNA and proteins can serve as an indication of cell damage. The adult cardiac muscle cell does not divide, and it may have a life span as long as that of entire organism (Rabinowitz, 1973; Fischman, Doyle and Zak, 1975). The constituents within the cell are in a state of equilibrium; they are constantly being destroyed and resynthesized. However, DNA synthesis is limited since there is no muscle cell multiplication, even though there are increases immediately following damage which are not well understood. The rate of uptake of radiolabelled nucleotides and amino acid analogues is directly related to increases in damaged muscle tissue and thus provides a technique for detecting and monitoring damage. This technique has

yet to be applied to the detection of damage in the myocardium by any more than one or two researchers (Arena et al., 1974; Rabinowitz, 1973) who have used mice and rats in their studies.

The introduction of phosphorus-31 nuclear magnetic resonance (^{31}PΔNMR) spectroscopy to the study of intact living tissues provides the capability of quantitatively analyzing the in vivo metabolism of discrete organophosphate metabolites without disrupting the integrity of the tissue under study. Since the initial demonstration of the potential biomedical applications of this methodology by Moon and Richards (1973), in their study of erythrocytes in whole blood, a variety of intact tissues has been analyzed, including perfused heart tissues. Phosphorus NMR spectra of perchloric acid (PCA) extracts prepared from these tissues have generated profiles qualitatively similar to those derived from the intact tissue, but with enhanced resolution and quantification precision. The sophistication of ^{31}PΔ NMR techniques has been improved to enable the detection of metabolic changes induced by various insults (e.g., ischemia, hypoxia) in the intact beating heart. The metabolites that are quantifiable in the intact heart include adenosine triphosphate, adenosine diphosphate, adenosine monophosphate, phosphoryl creatine, inorganic orthophosphate, nicotinamide adenine dinucleotide and the phosphrylated triose and hexose sugars.

The principal advantage of the NMR technique is that in the intact tissue, dynamic changes can be quantified during discrete time

intervals, and the metabolic effects induced by specific toxicants can be quantified with respect to changes in the metabolite concentrations and enzyme kinetics of the affected reactions. Subsequent tissue PCA extract preparation of these same tissues provides a detailed analysis of the minor organophosphate molecules present in the same tissue.

The ^{31}PΔNMR analytical procedures enable quantification of between 15 and 30 separate phosphate metabolites present in cardiac tissue within a short time increment (2 minutes in the intact tissue; 4 hours in the concentrated tissue PCA extract); however, the requisite procedures and equipment are highly sophisticated and are only used in research investigations. Future development of this technique and its ability to assess quickly biochemical alterations in intact tissue may make it a useful procedure in rapid screening (Kopp, 1980).

2.3.4 Cultured Heart Cells

Cultured heart cells are similar biochemically to intact muscle cells; consequently, they will manifest the same types of changes observed in intact cells. For instance, damaged cultured heart cells will leak enzymes into the culture media; accordingly, the activity of the culture media can then be examined for indications of damage in the cultured cells. Since heart cells are rich in lactic dehydrogenase (LDH) and in creatine phosphokinase (CPK), these two enzymes are typically measured as sensitive indicators or injury. In

addition, acid phosphatase and succinic dehydrogenase are infrequently determined. Acid phosphatase activity increases when lysosomal membranes are damaged, and succinate dehydrogenase is a measure of mitochondrial membrane damage. This monitoring is easily performed and can be done for many cultures at one time (Acosta, 1979).

When Nitro Blue tetrazolium (NBT) and phenazine methosulphate react with succinate dehydrogenase, microscopically-observable formazan granules are formed. When the structural integrity of the mitochondrial membranes is maintained, NBT and phenazine methosulphate are unable to react with succinate dehydrogenase in mitochondria in sufficient quantities to be readily observable microscopically (Acosta and Wenzel, 1975). This technique has been used to a limited extent to detect mitochondrial damage. Many cellular biochemical components are assayed in heart cell cultures. These include adenosine triphosphate (ATP), phosphoryl creatine (both related to energy production), myosin and total protein. The levels of DNA, RNA and protein synthesis are also monitored.

The monitoring of DNA, RNA, and protein synthesis is primarily used as an indication of the growth rate of cultured cells. These levels of synthesis tend to vary as cellular contact is made.

2.3.5 Tissue Explants and Perfused Heart Preparations

The most common techniques used in both tissue explants and perfused heart preparations examine the preparations for metabolic

changes, particularly those metabolic changes involved in energy metabolism. For this reason, glucose, lactate, adenosine monophosphate (AMP) and guanosine monophosphate (GMP) have been determined. The most common enzymes assayed in the perfusate are LDH and CPK. Calcium levels have been determined in the heart tissue in order to monitor calcium exchange rates with the perfusion media. Besides energy metabolism, lipid metabolism has been studied and the levels of DNA, RNA and protein synthesis have been determined in a few studies.

Nonetheless, the most useful biochemical monitoring techniques used to detect damage in the perfused heart and in tissue explants examine the in vitro perfusate for changes in the enzymes LDH and CPK. The other biochemical constituents are monitored primarily for research purposes, rather than for use in a screening program.

2.3.6 Summary

Damage to the heart tissue is associated with significant increases in serum enzyme levels. The most important are LDH and CPK. These enzyme assays have had important clinical application in the diagnosis of human cardiac disease, partly because of the inaccuracy associated with functional diagnostic techniques. In humans, the ranges of normal enzyme activities have been carefully determined; however, in animals, normal enzyme levels are not adequately known, but are determined (e.g., from control animals) for each individual

study. This general lack of normal values is a disadvantage in using enzyme monitoring techniques in a screening program.

There are substantial changes in tissue electrolyte levels in damaged heart tissue. The tissue electrolytes normally monitored include calcium, sodium, potassium and magnesium. Lehr has recently completed studies which indicate that tissue electrolyte changes are more sensitive to early myocardial damage than serum enzymes. When additional developmental studies are completed, tissue electrolyte monitoring may become useful in screening for cardiac damage.

The monitoring of DNA, RNA and protein synthesis as an indication of heart damage has only been used by a few investigators and would not currently be useful in a screening program.

Cultured heart cells are similar biochemically to intact muscle cells. Many biochemical constituents of cultured heart cells have been monitored; however, the most useful are LDH and CPK. Monitoring leakage of these two enzymes provides a sensitive indication of cellular damage. These two enzymes also provide the most useful tool for detecting damage in perfused hearts and tissue explants, even though many biochemical substances have been monitored in these two systems.

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3.0 CONCLUSIONS AND RECOMMENDATIONS

The testing techniques for the assessment of cardiotoxicity have been classified in three categories: morphological, functional and biochemical. The most common techniques are used to examine cardiovascular function; nevertheless, examination of structural and biochemical alterations is important in assessing cardiac damage. The *in vitro* systems (i.e., cultured heart cells, tissue explants and perfused heart preparations) have been used to describe cardiac mechanisms and are important in detecting cardiac damage.

All of the testing techniques examined, with the exception of the morphologic techniques, lack sophistication and the interpretation of results is difficult. None of the techniques are sufficiently developed or standardized to be used in a tiered screening program. At this time, tests can only be positioned in descending order of usefulness and potential application to a screening program.

The evaluation of individual tests is based on a number of considerations. The major considerations for the cardiovascular system are the validity of the measurement; the skills involved in making the measurement; the equipment necessary; the time needed to complete the tests; the sensitivity, accuracy and reproducibility of results; and the ability of the test to reflect cardiovascular damage. At the current stage of development of cardiovascular system testing, many

of these considerations can only be evaluated subjectively, while others cannot be addressed at all due to a lack of data.

3.1 Criteria Used in Evaluating Cardiovascular System Tests

The following criteria have been selected to evaluate each cardiovascular system test:

- whether the test is sufficiently developed to be reproducible in a screening program
- whether the test is sufficiently sensitive to detect early subtle forms of damage and to reflect the extent of damage to the system
- whether procedures and instrumentation are sufficiently uninvolved to enable technicians with minimum additional training to perform the test
- whether the test is terminal to the animals used
- the amount of time necessary to complete the test (i.e., days to a few weeks)

In evaluating tests, other criteria such as equipment costs and animal and maintenance costs would normally be considered. However, much of the equipment utilized for testing in the cardiovascular system is unique in design and construction, and requisite data concerning the other criteria are unavailable. Evaluation of the tests' state of development, the skill necessary to perform them, and the ease of performing them is based on discussion with researchers, and a review of their publications and other literature dealing with cardiovascular testing.

Many of the invasive techniques may damage the animal or the animal's heart so that re-use of animals is probably unrealistic

except for larger animals (e.g., monkeys), where the expense of the animals may necessitate re-using them. Where pressure transducers have been surgically implanted to measure cardiac functional parameters, the animals could conceivably be used for the screening of a number of substances as long as the heart is not damaged to the point of altering experimental results. In a large scale screening program, the expense of purchasing and maintaining larger animals may prohibit their use. Most tests would, therefore, be performed in smaller animals (e.g., mouse, rat, hamster, guinea pig, rabbit).

The use of tissue explants or cultured heart cells requires that the animals be terminated, although only a few animals are necessary to supply tissues and cells for these techniques. The perfused heart techniques require an animal be terminated for each procedure.

3.2 Evaluation of Cardiovascular Tests for Potential Application to a Screening Program

None of the cardiovascular tests sufficiently satisfy the criteria to be immediately useful in a screening program. A numerical assessment of the testing techniques based on the criteria previously mentioned is shown in Table 3-1. This numerical assessment was made subjectively following discussion with researchers using these techniques and following a review of the current literature.

The advantages and disadvantages of each testing technique are described below with a discussion of their potential application to a screening program. Some tests could be included in a screening program with additional development; other techniques are too complex

TABLE 3-1

EVALUATION OF CARDIOVASCULAR TESTING TECHNIQUES

TECHNIQUE	LEVEL OF DEVELOPMENT (ANIMALS)	LEVEL OF SKILL	SENSITIVITY IN DETECTING DAMAGE	REPEATABILITY	SOPHISTICATION OF EQUIPMENT	TERMINAL TO ANIMAL	TIME[1]	COST[2]	SUITABILITY FOR A SHORT-TERM SCREENING PROGRAM
o Morphological									
- Gross Inspection	5	4	3	4	2	Yes	1	1-2	3
- Light Microscopy	5	4	4	4	3-4	"	3	1-2	4
- Electron Microscopy	4	5	5	4	4-5	"	4	3	2
o Functional									
- Heart Rate	5	2	1	5	2	No	1	1	2
- Arterial Blood Pressure	4	2	2	4	2-3	"	1	1	2
- Left Ventricular Pressure	3	4	3	3	4-5	Yes/No	3-4	2-3	2
- Aortic Pressure	3	4	3	3	4-5	"	3-4	2-3	2
- Aortic Flow	3	4	3	3	4-5	"	3-4	2-3	2
- Cardiac Output	3	4	3	3	4-5	"	3-4	2-3	2
- Electrocardiography	3	4	3	4	3-4	No	1	1	4
o Biochemical									
- LDH (Serum)	4	3	4	4	3-4	No	1	1	3
- CPK (Serum)	4	3	4	4	3-4	"	1	1	3
- Ca, Mg, Na, K (Tissue)	2	4	5	Un	4	Yes	3	2-3	4
- DNA, RNA, Protein Synthesis	1	5	Un	Un	4	"	5	5	1
o Cultured Heart Cells									
- Morphologic Alterations	3	4	4	3	3-4	Yes	5	2-3	2
- Beating Activity	3	4	3	3	3-4	"	"	2-3	2
- Biochemical	3	3	4	4	3-4	"	"	1	4
- Cytotoxicity	3	3	4	4	3-4	"	"	2	3
o Perfused Heart Preparations	3	3	3	3	3	"	4	3	4
o Tissue Explants	1	4	Un	Un	3	"	5	Un	2

Scale: 1-poor or very low; 2-fair or low; 3-good or medium; 4-very good or high; 5-excellent or very high; Un-Unknown.

1. Time Scale
   1 < 1 hour
   2 1-4 hours
   3 1 day
   4 2-4 days
   5 1-5 weeks

2. Cost Scale
   1 < $100
   2 < $500
   3 < $1,000
   4 < $5,000
   5 > $5,000

and are beyond the scope of such a program. Table 3-2 lists a battery of tests recommended for a short-term screening program.

Cultured Heart Cells

This technique has many of the advantages of other *in vitro* screening techniques as well as some additional advantages because of the unique electrical and physiological properties of heart cells. The cells' morphology, growth rate, beating activity, electrical properties and biochemical mechanisms can be examined for cytotoxicity.

The difficulty of extrapolating experimental results from single-cell systems to whole organisms is a common disadvantage of cultured heart cells and other *in vitro* systems. Cell purity in heart-cell cultures is difficult to obtain and nonmuscle-cell overgrowth can interfere with the normal heart-cell function. The state of differentiation can be highly variable and must be determined for each new culture.

Heart cell cultures have only been used by a few investigators and the procedures lack standardization. Further development and standardization of this system will be necessary before it will be generally useful in a screening program; however, it is one technique that shows considerable potential for the toxicity screening of chemical substances.

TABLE 3-2

RECOMMENDED TESTS FOR A
BATCH SCREENING PROGRAM TO DETECT CARDIOVASCULAR DAMAGE

In Vitro Techniques	In Vivo Techniques[1]
• Cultured Heart Cells	• Functional
- Biochemical (LDH, CPK)	- Left Ventricular Pressure
- Morphologic Alterations	- Arterial Pressure
- Cyto toxicity	- Aortic Flow
	- Cardiac Output
• Perfused Heart Preparations	- Electrocardiography
- Biochemical (LDH, CPK)	• Biochemical
- Electrocardiography	- Serum LDH
- Contraction Force	- Serum CPK
- Coronary Flow	- Tissue Electrolytes (Na, Ca, Mg, K)
	• Morphological
	- Gross Description
	- Light Microscopy
	- Limited[2] Electron Microscopy

[1]The *in vivo* techniques would be done in rats or other small laboratory animals.

[2]The electron microscopy would only be done on those tissues requiring additional structural analysis (i.e., subtle forms of damage).

Morphological Techniques

Even though these techniques are reasonably rapid and simple to perform, histopathological analysis may be difficult and the interpretation of the results arduous and subject to dispute. Gross observation and microscopic histopathology are important for verification of results in a screening program, especially in the cardiovascular system, where early structural changes in the heart do not usually alter functional parameters. Therefore, the use of gross observation and light microscopy would have prominent application in a screening program. Electron microscopy is too involved for a screening program and it would need to be limited to those substances that show uncertain toxicity by other techniques.

Perfused Heart Preparations

This technique has the advantage of isolating an organ from the body while maintaining function so that functional parameters can be monitored. The parameters normally monitored are electrical, physiological and biochemical. A few have also examined the perfused heart for ultrastructural changes, although most investigators have limited their examination of the perfused heart to biochemical alterations in metabolism related to loss in contractile force. The perfused heart techniques will need to be developed further to evaluate their potential application to the screening of toxic chemicals. There is little baseline information available concerning normal values for the various functional parameters. Degenerative changes in the heart

following removal from the body need to be delineated. Some techniques, such as cooling the myocardium during the isolation procedure, may be used to minimize degenerative changes. The perfused heart technique may be too involved to be useful in a screening program. This will need to be determined as the technique is developed and baseline information is produced.

Electrocardiography

This technique has the advantage of being easy to perform and is not invasive. The cardiac electrical activity can be monitored from the surface of the body by simply placing electrodes on the surface of the skin. The major disadvantage is that electrocardiography seems to be insensitive to early cardiac damage. Electrocardiography is currently being evaluated for its potential in detecting early myocardial degenerative changes in small laboratory animals, possibly by modifying current monitoring techniques, or by monitoring parameters (e.g., conduction intervals) that are different from those traditionally monitored.

Functional Techniques

Functional parameters such as arterial blood pressure, left ventricular pressure, aortic flow, aortic pressure and contractile force are too insensitive to reflect early damage in the myocardium, and most monitoring is too difficult to perform to have prominent application in a screening program. The heart has sufficient reserve capacity that damage must be excessive before significant changes are

observed in the functional parameters. In addition, many of the procedures are invasive and require surgical manipulation of the animal. There is also little baseline information for any of the functional parameters in laboratory animals. For these reasons, functional monitoring in small animals, with the exception of electrocardiography, would not be useful in a screening program.

Biochemical Techniques

These measurements have had limited application in small laboratory animals for monitoring cardiovascular damage and could currently have some application in a screening program. Monitoring enzyme levels may prove more useful in the future, as enzyme monitoring in small animals is developed and normal baseline values are established. Both lactic dehydrogenase and creatine phosphokinase seem to be sensitive to early damage. However, they have yet to be monitored in feeding studies for more than just a few toxic substances. Current studies indicate that tissue electrolyte changes may be more sensitive to early damage than serum enzyme changes. Tissue electrolyte monitoring may have important application in the future screening of chemical substances.

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4.0 REFERENCES

Acosta, D. and D.G. Wenzel, 1974. "Injury Produced by Free Fatty Acids to Lysosomes and Mitochondria in Cultured Heart Muscle and Endothelial Cells." Atherosclerosis 20:417-426.

Acosta, D., D.G. Wenzel and J.W. Wheatley, 1974. "Beating Duration of Cultured Rat Heart Cells as Affected by Drugs and Other Factors." Pharmacological Research Communications 6(3):263-271.

Acosta, D. and D.G. Wenzel, 1975. "A Permeability Test for the Study of Mitochondrial Injury in In Vitro Cultured Heart Muscle and Endothelioid Cells." Histochemical Journal 7:45-56.

Acosta, D. and R. Chappell, 1977. "Cardiotoxicity of Diazepam in Cultured Heart Cells." Toxicology 8:311-317.

Acosta, D. and M. Puckett, 1977. "Ischemic Myocardial Injury in Culture Heart Cells: Preliminary Observations on Morphology and Beating Activity." In Vitro 13(12):818-823.

Acosta, D. and D. Anuforo, 1977-1978. "Acute Mitochondrial Toxicity of Caffeine in Cultured Heart Cells." Drug and Chemical Toxicology 1(1):19-24.

Acosta, D., M. Puckett and R. McMillin, 1978a. "Ischemic Myocardial Injury in Cultured Heart Cells: Leakage of Cytoplasmic Enzymes From Injured Cells." In Vitro 14(8):728-732.

Acosta, D., M. Puckett and R. McMillin, 1978b. "Ischemic Myocardial Injury in Cultured Heart Cells: In Situ Lysosomal damage." Experientia 10:1388-1389.

Acosta, D., 1979. Personal Communication, November 15, 1979.

Adams, L. and P. Cole, 1975. "A New Method for the Direct Estimation of Blood Oxygen Content." Cardiovascular Research 9:443-446.

Alexander, C.S., 1972. "Cobalt-beer Cardiomyopathy. A Clinical and Pathologic Study of Twenty-Eight Cases." American Journal of Medicine 53:395-417.

Amromin, G.D., H.L. Gildenhorn, R.D. Solomon, B.B. Nad Karni and M.L. Jacobs, 1964. "The Synergism of X-Ray and Cholesterol-fat on the Development of Coronary Artery Lesions." Journal of Atherosclerosis Research 4:325-335.

Arena, E., F. Biondo, N. D'Alessandro, L. Dusonchet, N. Gebbia, F. Gerbasi, L. Rausa and R. Sanguendoke, 1974. "DNA, RNA and Protein Synthesis in the Heart, Liver and Brain." IRCS (Research on: Biochemistry; Cancer; Pharmacology) 2:1053.

Arminger, L.C. and J.B. Gavin, 1975. "Changes in the Microvasculature of Ischemic and Infarcted Myocardium." Laboratory Investigation 33:51-56.

Aronson, C.E. and E.R. Serlick, 1977. "Effects of Prolonged Perfusion Time on the Isolated Perfused Rat Heart." Toxicology and Applied Pharmacology 38:479-488.

Aronson, C.E., E.R. Serlick and G. Preti, 1978. "Effects of Di-2-Ethylhexyl Phthalate on the Isolated Perfused Rat Heart." Toxicology and Applied Pharmacology 44:155-169.

Asford, T.P. and D.G. Freiman, 1967. "The Role of the Endothelium in the Initial Phases of Thrombossis: An Electron Microscopic Study." American Journal of Pathology 50:257-273.

Auclair, M.C. and C. Vernimmen, 1980. "Transmembrane Potential Recordings in Monolayer of New Born Ratheart Cells." Biologie Cellulaire 37:125-130.

Auger, C. and J. Chenaud, 1967. "Quebec Beer-Drinker's Cardiomyopathy: Ultrastructural Changes." Canadian Medical Association Journal 97:916-921.

Balazs, T., 1973. "Cardiotoxicity of Sympathominetic Bronchodilator and Vasodilating Anti-hypertensive Drugs in Experimental Animals." In: Experimental Model Systems in Toxicology and Their Significance in Man (Excerpta Medica, International Congress Series No. 311), Proceedings of the European Society for the Study of Drug Toxicity, pp. 71-79.

Balazs, T., F.L. Earl, G.W. Bierbower and M.A. Weinberger, 1973. "The Cardiotoxic Effects Aerosol Isoproterenol in the Dog." Toxicology and Applied Pharmacology 26:407-417.

Balazs, T.A. and E.H. Herman, 1976. "Toxic Cardiomyopathies." Annals of Veterinary Laboratory Science 6(6):467-476.

Balazs, T.A., E.H. Herman and F.L. Earl, 1976. "Myocardial Neurosis Induced by Diazoxide and Minoxidel in Dogs." In New Antihypertensive Drugs. Edited by Scriabine and Sweet, Spectrum Publish. Co., Hollinswood, N.J., pp. 535-547.

Bellamy, R.E., 1978. "Diastolic Coronary Artery Pressure-Flow Relations in the Dog." Circulation Research 43(1):92-101.

Bertazzoli, C., T. Chieli, G. Ferni, G. Ricevuti and E. Solcia, 1972. "Chronic Toxicity of Adriamycin: A New Antineoplastic Antibiotic." Toxicology and Applied Pharmacology 21:287-301.

Bigger, J.T., Jr. and C.C. Jaffe, 1971. "The Effect of Bretylium Tosylate on the Electrophysiologic Properties of Ventricular Muscle and Purkinje Fibers." American Journal of Cardiology 27:82-92.

Bloom, S. and D.L. Davis, 1972. "Calcium as Mediator of Isoproterenol Induced Myocardial Necrosis." American Journal of Pathology 69:459-470.

Blukoo-Allotey, J.A., N.H. Vincent and S. Ellis, 1969. "Interactions of Acetylcholine and Epinephrine on Contractility, Glycogen and Phosphorylase Activity of Isolated Mammalian Hearts." Journal of Pharmacology and Experimental Therapeutics 170(1):27-36.

Bonadonna, G. and S. Monfardini, 1969. "Cardiac Toxicity of Daunorubicin." Lancet 1:837.

Boor, P.J., M.T. Moslen and E.S. Reynolds, 1979. "Allylamine Cardiotoxicity: I. Sequence of Pathologic Events." Toxicology and Applied Pharmacology 50:581-592.

Booth, N.H., 1962. "Evaluation of Cardiac Monitor." Journal of American Veterinary Association 140:664-667.

Braunwald, E., S.J. Sarnoff and W.N. Stainsby, 1958. "Determinants of Duration and Mean Rate of Ventricular Ejection." Circulation Researh 6:319-325.

Brobmann, G.F., H.B. Ulano, G. Moore, P. Meyer, C.A. Brecher and E.D. Jacobsen, 1970. "Effect of Acute Hemorrhage on Arterial and Venous Resistance." Technical Report No. 25, University of Oklahoma Medical Center, For: Office of Naval Research, pp. 1-16.

Bult, H. and I.L. Bonta, 1976. "Prostaglandin Endoperoxides, Serotonin and the Superfused Rabbit Aorta: Possible Pitfalls in the Bioassay of Rabbit Aorta Contracting Substance (RCS)." Agents Actions 6(6):712-720.

Buja, L.M., V.J. Ferrans, R.J. Mayer, W.C. Roberts and E.S. Henderson, 1973. "Cardiac Ultrastructural Changes Induced by Daunorubicin Therapy." Cancer 32:771-788.

Buja, L.M., V.J. Ferrans and B.J. Maron, 1974. "Intracytoplasmic Junctions in Cardiac Muscle Cells." American Journal of Pathology 74:613-647.

Buja, L.M., V.J. Ferrans and A.S. Rabson, 1974. "Unusual Nuclear Alterations." Lancet 1:402-403.

Carmlet, S.H., H.H. Erickson, H.A. Gorman, 1975. "Ventricular Function Following Acute Carbon Monoxide Exposure." Journal Applied Physiology 39:482-486.

Caulfield, J.B., R.W. Shelton and J.F. Burke, 1972. "Cytotoxic Effects of Oxygen on Striated Muscle." Archives of Pathology 94:127-132.

Chalcroft, S.G.W., J.B. Gavin and P.B. Herdson, 1973. "Fine Structural Changes in RatMyocardium Induced by Daunorubicin." Pathology 5:99-105.

Chen, C.M., L.S. Gettes and B.G. Katzung, 1975. "Effect of Lidocaine and Quinidine on Steady-State Characteristics and Recovery Kinetics of (dv/dt) Max in Guinea Pig Ventricular Myocardium." Circulation Research 37:20-29.

Cheung, W.Y. and J.R. Williamson, 1965. "Kinetics of Cyclic Adenosine Monophosphate Changes in Rat Heart Following Epinephrine Administration." Nature 207:979-981.

Coodley, F.L., 1970. "Enzymes in Cardiac Disease." In: Diagnostic Enzymology. Edited by Coodley, Lea and Febiger, Philadelphia, Pennsylania, pp. 39-71.

Constantinides, P. and M. Robinson, 1969. "Effects of Vasoactive Amines." Archives of Pathology 88:106-112.

Cotran, R.S., 1967. "Delayed and Prolonged Vascular Leakage in Inflammation III, Immediate and Delayed Vascular Reactions in Skeletal Muscle." Experimental Molecular Pathology 6:143-155.

Cramlet, S.H., H.H. Erickson and H.A. Gorman, 1974. "Ventricular Function following Acute Carbon Monoxide Exposure." Journal of Applied Physiology 39(3):482-486.

Crill, W.E., R.E. Rumerg and J.W. Woodburg, 1959. "Effects of Membrane Current on Transmembrane Potentials of Cultured Chick Embryo Heart Cells." American Journal of Physiology 197:733-735.

Csapo, Z., J. Dusek and G. Rona, 1972. "Early Alterations of the Cardiac Muscle Cells in Isoproterenol-Induced Necrosis." Archives of Pathology 93:356-366.

D'Agostino, A.N., 1963. "An Electron Microscopic Study of Skeletal and Cardiac Muscle of the Rat Poisoned by Plasmocid." Laboratory of Investigation 12:1060-1071.

Davis, R.A. and K.C. Back, 1978. "The Effects of Bromochlorodifluoromethane and Mn^{2+} on Rate-Dependent Changes in Contractibility of Isolated Atria." Toxicology and Applied Pharmacology 45(1):227.

De Haan, R.L. and H.A. Fozzard, 1975. "Membrane Responses to Current Pulses in Spheroidal Aggregates of Embryonic Heart Cells." Journal of General Physiology 65:207-222.

Dhalla, N.S., J.C. Yates, S.L. Lee and A. Singh, 1978. "Functional and Subcellular Changes in the Isolated Rat Heart Perfused with Oxidized Isoproterenol." Journal of Molecular and Cellular Cardiology 10:31-41.

Dixon, J.S., J.R. Fozard and J.A. Gosling, 1978. "Atrial Sympathetic Nerves After Perfusion of the Rabbit Heart with Low Sodium Solutions Containing Potassium Chloride or Urea-A Biochemical, Histochemical and Fine Structural Study." Neuroscience 3:1157-67.

Dorigotti, L., M. Gaetani, A.H. Glasser and E. Turolla, 1969. "Competitive Antagonism of Isoprenatine-Induced Cardiac Necroses by -Adrenoreceptor Blocking Agents." Journal of Pharmacy and Pharmacology 21:188-191.

Dowell, R.T., A.F. Cutilletta and P.C. Sodt, 1975. "Functional Evaluation of the Rat Heart In Situ." Journal of Applied Physiology 39:1043-1047.

Drummond, G.I., L. Duncan and E. Hertzman, 1966. "Effects of Epinephrine on Phosphorylase b Kinase in Perfused Rat Hearts." Journal of Biological Chemistry 241:5899-5903.

Drummond, G.I. and S.J. Hemmings, 1973. "Role of Adenylate Cyclase-Cyclic AMP in Cardiac Actions of Adrenergic Amines." In: Recent Advances in Studies on Cardiac Structure and Metabolism, Volume 3. Edited by Dhalla, University Park Press, Baltimore, pp. 213-222.

Eikenburg, D.C. and J.L. Stickney, 1979. "Cardiac Effects of 1- -Acetylmethadol. V. Possible Involvement of the Autonomic Nervous System." Toxicology and Applied Pharmacology 51:91-100.

Fajardo, L.F. and J.R. Stewart, 1971. "Capillary Injury Preceding Radiation-Induced Myocardial Fibrosis." Radiology 101:429-433.

Fawcett, D.W., 1969. An Atlas of Fine Structure: The Cell, Its Organelles and Inclusions. W.B. Sanders, Philadelphia, pp. iii-v.

Ferguson, R.J., J.A. Faulkner, S. Julius and J. Conway, 1968. "Comparison of Cardiac Output Determined by CO_2 Rebreathing and Dye-Dilution Methods." Journal of Applied Physiology 25:450-454.

Ferrans, V.J., L.M. Buja and B.J. Maron, 1975. "Myofibrillar Abnormalities Following Cardiac Muscle Cell Injury: Pathophysiology and Morphology of Myocardial Cell Alteration." In: Recent Advances in Studies on Cardiac Structure and Metabolism, Volume 6. Edited by Fleckenstein and Rona, University Park Press, Baltimore, pp. 367-382.

Fleckenstein, A., J. Janke, H.J. Doring, and O. Pachinger, 1973. "Ca Overload as the Determinant Factor in the Production of Catecholamine-Induced Myocardial Lesions." In: Recent Advances in Studies on Cardiac Structure and Metabolism, Volume 2. Edited by Balazs and Rona, University Park Press, Baltimore, pp. 455-466.

Fleckenstein, A., J. Janke, H.J. Doring, and O. Leder, 1974. "Myocardial Fiber Necrosis Due to Intracellular Overload." In: Recent Advances in Cardiac Structure and Metabolism, Volume 4. Edited by Dhalla, University Park Press, Baltimore, pp. 563-580.

Fischman, D.A., C.M. Doyle and R. Zak, 1975. "DNA Synthesis in Chick Cardiac Muscle: Comparative Observations of In Vivo and In Vitro Growth." In: Perspectives in Cardiovascular Research - Developmental and Physiologic Correlates of Cardiac Muscle, Volume 1. Raven Press, New York, pp. 67-79.

Freeman, S.E., 1977. "Actions of Lidocaine on Atrial and Nodal Transmembrane Potentials in Guinea Pig and Rabbit." Materials Research Laboratories, Department of Defense, Ascot Vale, Victoria, Australia, pp. 1-20.

Freeman, S.E. and R.J. Turner, 1974. "Phase-Plane Trajectories of Arial Cell Action Potentials: Effects of Temperature Reduction." Cardiovascular Research 8:451-45[illegible].

Freer, R.J., A.J. Pappano, M.J. Peach, K.T. Bing, M.J. McLean, S. Vogel and N. Sperelakis, 1976. "Mechanism for the Positive Inotropic Effect of Angiotensin II on Isolated Cardiac Muscle." Circulation Research 39:178-183.

Friedman, P.L., J.R. Stewart, J.J. Feneglio and A.L. Wit, 1973. "Survival of Subendocardial Purkinje Fibers after Extensive Myocardial Infarction in Dogs: In Vitro and In Vivo Correlations." Circulation Research 33:597-611.

Gabbiani, G., M. Badonnel and G. Rona, 1975. "Cytoplasmic Contractile Apparatus in Aortic Endothelial Cells of Hypertensive Rats." Laboratory Investigation 32:227-234.

Gad, S., S.W. Leslie and D. Acosta, 1979. "Inhibitory Actions of Butylated Hydroxytoluene on Isolated Ileal, Atrial, and Perfused Heart Preparations." Toxicology and Applied Pharmacology 49:45-52.

Gardner, R.M. and D.O. Allen, 1977. "The Relationship Between Cyclic Nucleotide Levels and Glycogen Phosphorylase Activity in Isolated Rat Hearts Perfused with Epinephrine and Acetylcholine." Journal of Pharmacology and Experimental Therapeutics 202(2):346-353.

George, W.J., J.B. Polson, A.G. O'Toole and N.D. Goldberg, 1970. "Elevation of Guanosine 3',5'-Cyclic Phosphate in Rat Heart After Perfusion with Acetylcholine." Proceedings of the National Academy of Sciences 66:398-403.

Gould, K.L., K. Lipscomb and G.W. Hamilton, 1974. "Physiologic Basis for Assessing Critical Coronary Stenosis. Instantaneous Flow Response and Regional Distribution During Coronary Hyperemia as Measures of Coronary Flow Reserve." American Journal of Cardiology 33:87.

Goshima, K., 1975. "Beating of Myocardial Cells in Culture." In: Developmental and Physiological Correlates of Cardiac Muscle. Edited by Lieberman and Sano, Raven Press, New York, pp. 197-208.

Goshima, K., 1977. "Quabain Induced Arrhythmias of Single Isolated Myocardial Cells and Cell Clusters Cultured In Vitro, and Their Improvement by Quinidine." Journal of Molecular Cellular Cardiology 9:7-23.

Hall, J.L. and E.B. Smith, 1968. "Cobalt Heart Disease: An Electron Microscopic and Histochemical Study in the Rabbit." Archives of Pathology 86:403.

Handforth, C.P. and N.S. Halifax, 1962. "Isoproternol-Induced Myocardial Infarction in Animals." Archives of Pathology 73:161-165.

Harary, J., A. Fujimoto and H. Kuramitsu, 1964. "Enzyme Changes In Cultured Heart Cells." National Cancer Institute Monographs 13:257-275.

Hearse, D.J., S.M. Humphrey, D. Feuvray, and J.A. Deleiris, 1976. "A Biochemical and Ultrastructural Study of the Species Variation in Myocardial Cell Damage." Journal of Molecular Cardiology 8:759-788.

Heggtveit, H.A., 1965. "The Cardiomyopathy of Magnesium-Deficiency." In: Electrolytes and Cardiovascular Disease. Edited by Bajusz, Volume 1, S. Kavager, New York, pp. 204-221.

Heggtveit, H.A. and B.B. Nadkarni, 1971. "Ultrastructural Pathology of the Myocardium." In: Methods and Achievementsin Experimental Pathology. Edited by Bajusz and Jasmin, Volume 5, S.Karger, New York, pp. 474-517.

Herdson, P.B., H.M. Sommers and R.B. Jennings, 1965. "A Comparative Study of the Fine Structure of Normal and Ischemic Dog Myocardium with Special Referenceto Early Changes Following Temporary Occlusion of a CoronaryArtery." American Journal of Pathology 46:367-386.

Herdson, P.B., J.P. Kaltenbachand and R.B. Jennings, 1969. "Fine Structural and Biochemical Changes in Dog Myocardium During Autolysis." American Journal of Pathology 57:539-557.

Herman, E.H. and J.A. Vick, 1970. "The Acute Pharmacological Actions of Daunomycin in the Dog and Monkey." Pharmacology 3:291-304.

Hessler, J.R., A.L. Robertson and G.M. Chisolm, 1979. "LDL-Induced Cytotoxicity and Its Inhibition by HDL in Human Vascular Smooth Muscle and Endothelial Cells in Culture." Atherosclerosis 32:213-229.

Hibbs, R.C., V.J. Ferrans, W.C. Black, D.G. Weilbaecher, J.J. Walsh and G.E. Burch, 1965. "Alcoholic Cardiomyopathy: An Electron Microscopic Study." American Heart Journal 69:766-779.

Hinshaw, L.B., L.T. Archer, L.J. Greenfield, C.A. Guenter, and J.A. Miller, 1970. "Absence of a Direct Toxic Action of Endotoxin on Myocardial Tissue." Technical Report No. 36, University of Oklahoma Medical Center, For: Office of Naval Research, Washington, D.C., pp. 1-22.

Hinshaw, L.B., L.T. Archer, L.J. Greenfield, C.A. Guenter, 1971. "Effects on Endotoxin on Myocardial Hemodynamics, Performance and Metabolism." Technical Report No. 39, University of Oklahoma Medical Center, For: Office of Naval Research, Washington, D.C., pp. 1-25.

Hinshaw, L.B., L.J. Greenfield, S.E. Owen, M.R. Black and C.A. Guenter, 1972a. "Precipitation of Cardiac Failure in Endotoxin Shock." Technical Report No. 50, University of Oklahoma Medical Center, For: Office of Naval Research, Washington, D.C., pp. 1-17.

Hinshaw, L.B., L.J. Greenfield, S.E. Owen, L.T. Archer, and C.A. Guenter, 1972b. "Cardiac Response to Circulating Factors in Endotoxin Shock." Technical Report No. 51, University of Oklahoma Medical Center, For: Office of Naval Research, Washington, D.C., pp. 1-11.

Horn, R.S., R. Levin and N. Haugaard, 1969. "The Influence of Oligomycin on the Actions of Epinephrine and Theophylline Upon the Perfused Heart." Biochemical Pharmacology 18:503-509.

Huttner, I., H. Jellinek and T. Kerenyi, 1968. "Fibrin Formations in Vascular Fibrinoid Change in Experimental Hypertension." Experimental and Molecular Pathology 9:309-321.

Hurst, J.W., R.B. Logue, R.C. Schlant and N.K. Wenger (eds.), 1978. The Heart, Arteries and Veins. Fourth Edition, McGraw-Hill, New York, pp. 19-70, 491-501.

Imai, S., Y. Katano, M. Nakazawa and N. Shimamoto, 1978. "The Effects of Norepinephrine on the Relase of Cyclic AMP by the Isolated Perfused Heart of the Guinea Pig." Life Sciences 23:1609-1618.

Ito, Y. and C.A. Chidsey, 1972. "Intracellular Calcium and Myocardial Contractility: IV. Distribution of Calcium in the Failing Heart." Journal of Molecular and Cellular Cardiology 4:507-517.

Jennings, R.B., J.H. Baum and P.B. Herdson, 1965. "Fine Structural Changes in MyocardialIschemic Injury." Archives of Pathology 79:135-143.

Jorgensen, L., H.C. Rowsell, T. Howig and J.F. Mustard, 1967. "Resolution and Organization of Platelet-Rich Mural Thrombi In Carotid Arteries of Swine." American Journal of Pathology 51:681-719.

Jorgensen, L., T. Hovig, H.C. Rowsell and J.F. Mustard, 1970. "Adenosine Diphosphate-Induced Platelet Aggregation and Vascular Injury in Swine and Rabbits." American Journal of Pathology 61:161-176.

Josephson, I., J.F. Renaud, S. Vogel, M. McLean and N. Sperelakis, 1976. "Mechanism of the Histamine-Induced Positive Inotropic Action in Cardiac Muscle." European Journal of Pharmacology 35:393-398.

Kahn, R.A., S.A. Johnson and A.F. DeGraff, 1971. "Effects of Sodium Warfarin on Capillary Ultrastructure." American Journal of Pathology 65:149-156.

Kallfelt, B.J., A.P. Waldenstrom and A.C. Hjalmarson, 1977. "Effects of Adrenaline In Vivo on Protein Synthesis and Sensitivity to Ischemia of the Perfused Rat Heart." Journal of Molecular and Cellular Cardiology 9:383-398.

Karsten, U., A. Kossler, E. Janiszewski and A. Wollenberger, 1973. "Influence of Variations in Pericellular Oxygen Tension on Individual Cell Growth, Muscle-Characteristic Proteins, and Lactate Dehydrogenase Isoenzyme Patterns in Cultures of Beating Rat Heart Cells." In Vitro 9(3):139-146.

Kopp, S.J., 1980. Personal Communication with R. Thomas of the MITRE Corporation on 15 September 1980.

Kopp, S.J., J.C. Baker, L.S. D'Agrosa and P.L. Hawley, 1978. "Simultaneous Recording of His Bundle Electrogram, Electrocardiogram, and Systolic Tension from Intact Modified Langendorff Rat Heart Preparations. I: Effects of Perfusion Time, Cadmium and Lead." Toxicology and Applied Pharmacology 46:475-487.

Langer, G.A. and J.S. Frank, 1975. "Calcium Exchange in Cultured Cardiac Cells." In: Perspective in Cardiovascular Research - Developmental and Physiologic Correlates of Cardiac Muscle, Volume 1. Edited by Lieberman and Sano, Raven Press, New York, pp. 117-126.

Lazzara, R., N. El-Sherif and B.J. Scherlag, 1973. "Electrophysiological Properties of Canine Purkinje Cells in One-Day-Old Myocardial Infarction." Circulation Research 33:722-734.

Lazzara, R., N. El-Sherif, B. Befeler and B.J. Scherlag, 1976. "Regional Refractoriness Within the Ventricular Conducting System: An Evaluation of the 'Gate' Hypothesis." Circulation Research 39:254-262.

Lefrak, E.A., J. Pitha, S. Rosenheim and J.A. Gottlieb, 1973. "A Clinicopathologic Analysis of Adriamycin Cardiotoxicity." Cancer 32:302-314.

Lehr, D., M. Krnkowski and R. Colon, 1966. "Correlation of Myocardial and Renal Necrosis with Tissue-Electrolyte Changes." Journal of the American Medical Association 197:145-152.

Lehr, D., 1969. "Tissue Electrolyte Alterations in Disseminated Myocardial Necrosis." Annals of the New York Academy of Sciences 156:344-378.

Lehr, D. and R. Chau, 1973. "Changes of Cardiac Electrolyte Content During Development and Healing of Experimental Myocardial Infarction." In: Recent Advances in Studies on Cardiac Structure and Metabolism, Volume III. Edited by Dhalla and Rona, University Park Press, Baltimore, pp. 721-751.

Lehr, D., R. Chau and S. Irene, 1975. "Possible Role of Magnesium Loss in the Pathogenesis of Myocardial Fiber Necrosis." In Pathophysiology and Morphology of Myocardial Cell Alteration, A. Fleckenstein and G. Rona (eds.). University Park Press, Baltimore.

Lehr, D., 1980. Personal Communication, 22 January 1980.

Leslie, S.W., S.C. Gad and D. Acosta, 1978. "Cytotoxicity of Butylated Hydroxytoluene and Butylated Hydroxyanisole in Cultured Heart Cells." Toxicology 10:281-289.

Liu, C.T. and R.P. Williams, 1971. "Presence of Cardioactive Substances in Cultures of Bacillus Anthracis." Archives Internationales de Pharmacodynamie et de Therapie 189:336-347.

Liu, C.T., 1976. "Cardiovascular and Renal Functions in Normal Rhesus Macaques." American Journal of Veterinary Research 37(8):969-974.

Liu, C.T., 1977. "Comparison of Cardio-Renal Functions between Women and Female Rhesus Macaques." Journal of American Veterinary Research 38(9):1439-1442.

Lubacsko, A.B., 1979. "On the Pathogenesis, Treatment and Prevention of Adrenergic Amine-Induced Myocardial Injury in the Albino Rat." Thesis for Department of Pharmacology New York Medical College, Graduate School of Basic Medical Sciences, Valhalla, New York.

Lubbe, W.F., M.L. McFadyen, C.A. Muller, M. Worthington and L.H. Opie, 1979. "Protective Action of Amiodarone Against Ventricular Fibrillation in the Isolated Perfused Rat Heart." American Journal of Cardiology 43(3):533-540.

Lundy, P.M., 1978. "Mechanism of the Cardiovascular Activity of Dibenzoxazepine in Cats." European Journal of Pharmacology 48(3):271-279.

Maher, J.T., J.C. Dennison, D.L. Wolfe and A. Cymerman, 1977. "Mechanism of the Attenuated Cardiac Response to Beta-Adrenergic Stimulation in Chronic Hypoxia." U.S. ArmyResearch Institute of Environmental Medicine, Natick, MA, pp. 1-20.

McGrath, J.J., K.C. Chen and J.J. Vostal, 1978. "Comparative Effects of Nitrogenand Carbon Monoxide on the Isolated Rat Heart." Presentation at Society of Toxicology, March 12-16, 1978.

McKay, D.G., M.M. Linder and V.K. Cruse, 1971. "Mechanisms of Thrombosis of the Microcirculation." American Journal of Pathology 63:231-254.

McNeill, J.H., R.V. Barnes, R.S. Davis, and J.B. Hook, 1969. "The Effects of Vasodilator-Drugs on the Noradrenaline Constrictor Response in the Isolated Mesenteric Artery." Canadian Journal of Physiology and Pharmacology 47:663-669.

Mettler, F.P. D.M. Young, and J.M. Ward, 1977. "Adriamycin-induced Cardiotoxicity (Cardiomyopathy and Congestive Heart Failure) in Rats." Cancer Research 37:2705-2713.

Molnar, Z., K. Larsen and B. Spargo, 1962. "Cardiac Changes in the Potassium-Depleted Rat." Archives of Pathology 74:339-347.

Moon, R.B. and J.H. Richards, 1973. "Determination of Intracellular by pH by ^{31}P Magnetic Resonance." Journal of Biological Chemistry 248:7276-7278.

Morin, Y. and Cote, G., 1972. "Toxic Agents and Cardiomiopathies." In: Cardiomyopathy. Edited by Burch. Cardiovascular Clinics 4:245-267.

Morrison, A.D., L. Orci, L. Berwick, A. Perrelet and A.I. Winegrad, 1977. "The Effects of Anoxia on the Morphology and Composite Metabolism of the Intact Aortic Intima-Media Preparation." Journal of Clinical Investigation 59:1027-1037.

Morvai, V. and G. Ungvary, 1979. "Effects of Simultaneous Alcohol and Toluene Poisoning on the Cardiovascular System of Rats." Toxicology and Applied Pharmacology 50:381-389.

Nayler, W.G., I. McInnes, J.B. Swann, D. Race, V. Carson and T.E. Lowe, 1968. "Some Effects of the Hypotensive Drug Diazoxide on the Cardiovascular System." American Heart Journal 75:223-232.

Olson, H.M., D.M. Young, D.J. Prieur, A.F. LeRoy, and R.L. Reagan, 1974. "Electrolyte and Morphologic Alterations of Myocardium in Adriamycin-Treated Rabbits." American Journal of Pathology 77:439-454.

Olson, H.M. and C.C. Capen, 1978. "Chronic Cardiotoxicity of Doxorubicin (Adriamycin) in the Rat: Morphologic and Biochemical Investigations." Toxicology and Applied Pharmacology 44:605-616.

Page, J.G., H.R. Sullivan, S.L. Due and I.H. Slater, 1979. "Plasma Concentrations and Electrocardiographic Alterations after Repetitive Administration of Propoxyphene to Dogs." Toxicology and Applied Pharmacology 50:505-514.

Pappano, A.J. and M. Sperelakis, 1969a. "Spontaneous Contractions of Cultural Heart Cells in High K^+ Media." Experimental Cell Research 54:58-68.

Pappano, A.J. and M. Sperelakis, 1969b. "Spike Electrogenesis in Cultured Heart Cells." American Journal of Physiology 217: 615-624.

Pasyk, S., C.M. Bloor, E.M. Khouri and D.E. Gregg, 1971. "Systemic and Coronary Effects of Coronary Artery Occlusion in the Unanesthetized Dog." American Journal of Physiology 220:646-654.

Penney, D.G., P.C. Sodt and A. Cutilletta, 1979. "Cardiodynamic Changes During Prolonged Carbon Monoxide Exposure in the Rat." Toxicology and Applied Pharmacology 50:213-218.

Porter, M.C., T.S. Miya and W.F. Bousquet, 1975. "Cadmium and Vascular Reactivity in the Rat." Toxicology and Applied Pharmacology 34:143-150.

Powell, W.J., R.M. Green, R.B. Whiting, and C.A. Sanders, 1971. "Action of Diazoxide on Skeleted Muscle Vascular Resistance." Circulation Research 28:167-178.

Price, A.M., D.C. Pease and C.M. Pearson, 1962. "Selective Actinifilament and Z-band Degeneration Induced by Plasmocid." Laboratory Investigation 11:549-562.

Rabinowitz, M., 1973. "Protein Synthesis and Turnover in Normal and Hypertrophied Heart." American Journal of Cardiology 31:202-210.

Rabkin, S.W., J.M. Friesen, J.A. Ferris, and H.Y Fung, 1979. "A Model of Cardiac Arrhythmias and Sudden Death Cantharidin induced Toxic Cardiomyopathy." Journal of Pharmacology and Experimental Therapeutics 210(1):43-50.

Regan, T.J., C.A. Daniels, T. Borsos, R. Snyderman and A.L. Notkins, 1969. "Ventricular Arrhythmias Related to Antibiotic Usage in Dogs." Science 165:509-511.

Reichenbach, D.D. and E.P. Benditt, 1970. "Catecholamines and Cardiomyopathy: the Pathogenesis and Potential Importance of Myofibrillar Degeneration." Human Pathology 1:125-150.

Robinson, G.A., R.W. Butcher, I. Øye, H.E. Morgan and E.W. Sutherland, 1965. "The Effect of Epinephrine on Adenosine 3',5'-Phosphate Levels in the Isolated Perfused Rat Heart." Molecular Pharmacology 4:168-177.

Rona, G., C.I. Chappel, T. Balazs and R. Gaudry, 1959. "An Infarct-like Myocardial Lesion and Other Toxic Manifestation Produced by Isoproterenol in the Rat." Archives of Pathology 67:443-445.

Rona, G., D.S. Kahn and C.I. Chappel, 1963. "Studies on Infarct-like Myocardial Necrosis Produced by Isoproterenol." Reviews Canadian Biology 22:241-255.

Rona, G. and C.I. Chappel, 1973. "Pathogenesis and Pathology of Cobalt Cardiomyopathy." In: Recent Advances in Studies of Cardiac Structure and Metabolism, Volume 2. Edited by Bajusz, University Park Press, Baltimore, pp. 407-422.

Rosenoff, S.H., H.M. Olson, D.M. Young, F. Bostick and R.C. Young, 1975. "Adriamycin-induced Cardiac Damage in the Mouse: A Small-Animal Model of Cardiotoxicity." Journal of the National Cancer Institute 55:191-194.

Rosenthal, M.D. and J.B. Warshaw, 1973. "Interaction of Fatty-Acid and Glucose Oxidation by Cultured Heart Cells." Journal of Cell Biology 58:332-339.

Ross, B.D., 1972. Perfusion Techniques in Biochemistry. Chap. 5. Oxford Univesity Press, London, pp. 258-320.

Rowe, G.G., T.R. Leicht, W.C. Boake, J.C. Kyle and C.W. Crumpton, 1963. "The Systemic and Coronary Hemodynamic Effects of Diazoxide." American Heart Journal 66:636-643.

Schneider, J.A. and N. Sperelakis, 1974. "The Demonstration of Energy Dependence of the Isoproterenol Induced Transcellular Ca^{2+} Current in Isolated Perfused Guinea Pig Hearts, An Explanation for Mechanical Failure of Ischemic Myocardium." Journal of Surgical Research 16:389-403.

Schneider, J.A. and N. Sperelakis, 1975. "Slow Ca^{2+} and Na^{+} Responses Induced by Isoproterenol and Methylxanthines in Isolated Perfused Guinea Pig Hearts Exposed to Elevated K^{+}." Journal of Molecular and Cellular Cardiology 7:249-273.

Scott J.C. and A.W. Cowley, 1969. "The Effect of Diazoxide on Coronary Blood Flow." American Journal of Cardiology 24:865-869.

Selye, H., 1961. The Pluricausal Cardiopathies. Charles C. Thomas, Springfield, IL.

Serabdarian, M.W., L. Artaza and M.F. Goodman, 1977. "Adriamycin: Effect on Mammalian Cardiac Cells in Culture. I. Cell Population and Energy Metabolism." Journal of Molecular and Cellular Cardiology 9:375-382.

Shen, A.C. and R.B. Jennings, 1972. "Myocardial Ca and Mg in Acute Ischemic Injury." American Journal of Pathology 67:417-440.

Sperelakis, N. and D. Lehmkahl, 1965. "Insensitive of Cultured Chick Heart Cells to Autonomic Agents and Tetrodotoxin." American Journal of Physiology 209:693-698.

Sperelakis, N. and D. Lehmkahl, 1968. "Ba^{2+} and Sr^{2+} Reversal of the Inhibition Produced by Quabain and Local Anesthetics on Membrane Potentials of Cultured Heart Cells." Experimental Cell Research 49:396-410.

Sperelakis, N. and K. Shigenobu, 1974. "Organ-Cultured Chick Embryonic Hearts of Various Ages: I. Electrophysiology." Journal of Molecular and Cellular Cardiology 6:449-471.

Sperelakis, N. and K. Shigenobu, 1974. Organ-Cultured Chick Embryonic Hearts of Various Ages: II. Ultrastructure." Journal of Molecular and Cellular Cardiology 6:473-483.

Sperelakis, N. and M.J. McLean, 1978. "Electrical Properties of Cultured Heart Cells." In: Recent Advances in Studies on Cardiac Structure and Metabolism, Volume 12. Edited by: Kobayashic, Ito and Rona, University Park Press, Baltimore, pp. 645-666.

Stanton, H.C. and J.B. White, Jr., 1965. "Hypotensive Actions of Drugs on Unanesthetised Normotensive and 'Metacorticoid' Hypertensive Rats Determined by a Direct Recording Technique." Archives Internationales de Pharmacodynamie et de Therapie 154:351-363.

Stearner, S.P. and E.J.B. Christian, 1971. "Mechanisms of Acute Injury in the -Irradiated Chicken In Vivo Studies of Dose Protraction Effects on the Microvasculature." Radiation Research 47:741-755.

Still, W.J.S. and G.B.D. Scott, 1966. "An Electron Microscopic Study of the Endothelial Changes and the Nature of 'Fibrinoid' Produced in the Generalized Shwartzman Reaction." Experimental Molecular Pathology 5:118-124.

Stong, L.J., C.R. Hartzell and R.L. McCarl, 1975. "Halothane and the Beating Response and ATP Turnover Rate of Heart Cells in Tissue Culture." Anesthesiology 42(2):123-132.

Su, J.Y. and C. Wei-jen, 1979. "The Effects of Methylmercury on Isolated Cardiac Tissues." The American Journal of Pathology 95(3): 753-764.

Sulkin, N.M. and D.F. Sulkin, 1965. "An Electron Microscopic Study of the Effects of Chronic Hypoxia on Cardiac Muscle, Hepatic, and Autonomic Ganglion Cells." Laboratory Investigation 14:1523-1546.

Susin, M. and P.B. Herdson, 1967. "Fine Structural Changes in Rat Myocardium Induced by Thyroxine and by Magnesium Deficiency." Archives of Pathology 83:86-98.

Tacker, W.A., J.F. Van Vleet and L.A. Geddes, 1979. "Electrocardiographic and Serum Enzymic Alterations Associated with Cardiac Alterations Induced in Dogs by Single Transthoracic Damped Sinusoidal Defibrillator Shocks of Various Strengths." American Heart Journal 98(2):185-193.

Takenaka, F. and S. Takeo, 1976. "Effects of Isoproterenol on Myocardial Lipid Metabolism in Rat Hearts Perfused With and Without Exogenous Substrates." Journal of Molecullar and Cellular Cardiology 8:925-940.

Tanz, R. and L. Opie, 1978. "Effect of Drug or Electrically Induced Tachy Arrhythmias on the Release of Lactate Dehydrogenase (LDH) in Isolated Perfused Guinea-Pig Hearts. I. Comparison of the Effects Produced by Quabain, Calcium, Epinephrine and Aconitine." Journal of Pharmacology and Experimental Therapeutics 206(2):320-330.

Taylor IV, G.J. and R.T. Drew, 1975. "Cardiovascular Effects of Acute and Chronic Inhalations of Fluorocarbon 12 in Rabbits." Journal of Pharmacology and Experimental Therapeutics 192:129-135.

Thind, G.S., G. Karreman, K.F. Stephan and W.S. Blakemore, 1970. "Vascular Reactivity and Mechanical Properties of Normal and Cadmium-Hypertensive Rabbits." Journal of Laboratory Clinical Medicine 76:560-568.

Tzivoni, D., R. Yarcom, M. Fields, T.A. Hall and M.S. Gotsman, 1978. "X-ray Microanalysis of Isolated Perfused Rat Hearts." Basic Research in Cardiology 73:200-207.

Vanarsdel, W.C., 1979. Electrocardiographic Criteria for Detection of Cardiac Damage. Toxicology Research Projects Directory 4(2):1-50.

Van Vleet, J.F., V.J. Ferrans and G.R. Ruth, 1977a. "Ultrastructural Alterations in Nutritional Cardiomyopathy of Selenium-Vitamin E Deficient Swine, I. Fiber Lesions." Laboratory Investigation 37:188-200.

Van Vleet, J.F., V.J. Ferrans and G.R. Ruth, 1977b. "Ultrastructural Alterations in Nutritional Cardiomyopathy of Selenium-Vitamin E Deficient Swine. II. Vascular Lesions." Labortory Investigation 37: 201-211.

Van Vleet, J.F., V.J. Ferrans and W.E. Weirich, 1980. "Cardiac Disease Induced by Chronic Adriamycin Administration in Dogs and an Evaluation of Vitamin E and Selenium as Cardioprotectants." The American Journal of Patholoy 99(1):13-42.

Vatner, S.F., 1978. "Effects of Anesthesia on Cardiovascular Control Mechanisms." Environmental Health Perspectives 26:193-206.

Vernimmen, C. and M.C. Auclair, 1980. "Electro-Optical Technique for Recording Contractile Activity of Cultured Heart Cells." Biologie Cellulaire 37:143-148.

Vincent, N.H. and S. Ellis, 1963. "Inhibitory Effect of Acetylcholine on Glycogenolysis in the Isolated Guinea-Pig Heart." Journal of Pharmacology and Experimental Therapeutics 139:60-68.

Walsh, G.M., M. Tsuchiya and E.D. Frohlich, 1976. "Direct Fick Application for Measurement of Cardiac Output in Rat." Journal of Applied Physiology 40:849-853.

Wastila, W.B., J.Y. Su, W.F. Friedman and S.E. Mayer, 1972. "Blockade of Biochemical and Physiological Responses of Cardiac Muscle to Norepinephrine by N-Terf-Butylmethoxamine (Butoxamine)." Journal of Pharmacology and Experimental Therapeutics 181(1):126-138.

Wennmalm, A., 1977. "Nicotine Stimulates Prostagland Formation in the Rabbit Heart." British Journal of Pharmacology 59(1):95-100.

Wennmalm, A., 1978. "Nicotine Inhibits the Release of 6-Keto-Prostaglandlin F-1-alpha from the Isolated Perfused Rabbit Heart. Acta Physiologica Scandinavica 103:107-109.

Wenzel, D.G. and D. Acosta, 1973. "Permeability of Lysosomes and Mitochondria in Cultured Rat Heart Muscle and Endothelioid Cells as Affected by Vitamin A, Chloropromazine, Amphotericin B, and Clofibrate." Research Communications in Chemical Pathology and Pharmacology 6(2):689-700.

Wenzel, D.G. and D. Acosta, 1976. "The Use of Cultured Cells for In Situ Measurement of Mitochondrial Fragility." Reprint from: The Tissue Culture Association, pp. 221-223.

Wenzel, D.G. and J.W. Kleoppel, 1978. "Arrhythmias Induced by Changing the Medium of Cultured Rat Heart Muscle Cells: A Model for Assessment of Antiarrhythmic Agents." Journal of Pharmacological Methods 1:269-276.

Wiedmeier, V.T. and L.H. Spell, 1977. "Effects of Catecholamines, Histamine, and Nitroglycerin on Flow, Oxygen Utilization, and Adenosine Production in the Perfused Guinea Pig Heart." Circulation Research 41(4):503-508.

Wildenthal, K., 1971. "Long-Term Maintenance of Spontaneously Beating Mouse Hearts in Organ Culture." Journal of Applied Physiology 30:153-157.

Williamson, J.R., 1964. "Metabolic Effects of Epinephrine in the Isolated, Perfused Rate Heart." Journal of Biological Chemistry 239(9):2721-2729.

Willms-Kretschmer, K. and G. Majno, 1969. "Ischemia of the Skin, Electron Microscopic Study of Vascular Injury." American Journal of Pathology 54:327-353.

Wit, A.L., C. Steiner and A.M. Damato, 1970. "Electrophysiological Effects of Bretylium Tosylate on Single Fibers of the Canine Specialized Conducting System and Ventricle." Journal of Pharmacology and Experimental Therapeutics 173:344-356.

Wohl, A.J., L.M. Hausler and F.E. Roth, 1968b. "Mechanism of the Antihypertensive Effect of Diazoxide: In Vitro Vascular Studies in the Hypertensive Rat." Journal of Pharmacology and Experimental Therapeutics 162:109-114.

Wolfe, L.A., D.A. Cunningham, G.M. Davis and P.A. Rechmitzer, 1978. "Reliability of Noninvlasive Methods for Measuring Cardiac Function in Exercise." Journal of Applied Physiology 44(1):55-58.

Zech, W.H., J.H. Boucher, D.E. Hilmas and R.O. Spertzel, 1974. "Mycardial Contractility in Conscious Rhesus Monkeys: A Method of Long-Term Study." American Journal of Veterinary Research 35(1):83-86.

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APPENDIX A

MORPHOLOGICAL MEASUREMENTS OF MYOCARDIAL DAMAGE

TABLE A-1

MORPHOLOGICAL MEASUREMENTS OF MYOCARDIAL DAMAGE

SPECIFIC MEASUREMENTS	SPECIES OF ANIMALS USED	SUBSTANCES TESTED AND/OR CONDITION	REFERENCES	COMMENTS
GROSS APPEARANCE LIGHT, TRANSMISSION ELECTRON AND SCANNING ELECTRON MICROSCOPY, FLUORESCENCE MICROSCOPY	MICE, RATS, HAMSTERS, GUINEA PIGS, RABBITS, CATS, DOGS, PIGS, HUMANS	ISOPROTERENOL, ISOPRENALINE, PROPRANOLOL, PHENYLEPHRINE, EPINEPHRINE, METARAMINOL, POTASSIUM, MAGNESIUM AND SELENIUM DEFICIENCY, HYPOXIA (ISCHEMIA), ELECTRIC SHOCK, HYPERTENSION, HYPOTHERMIA, CORONARY ARTERY OCCLUSION, ADRIAMYCIN, DAUNORUBICIN, ALCOHOL, COBALT, PLASMOCID, ENDOTOXIN, 3-AMINOPROPENE, DIALOXIDE, MINOXIDIL, MALNUTRITION AND VITAMIN DEFICIENCIES	ALEXANDER, 1972; AUGER AND CHENAUD, 1967; BALAZS, 1973; BALAZS AND HERMAN, 1976; BALAZS, HERMAN AND EARL, 1976; BERTAZZOLI ET AL., 1972; BONADONNA AND MONFARDINI, 1969; BOOR, MOSLEN AND REYNOLDS, 1979; BUJA ET AL., 1973; BUJA, FERRANS AND MARON, 1974; BUJA, FERRANS AND RABSON, 1974; CAULFIELD, SHELTON AND BURKE, 1972; CHALCROFT, GAVIN AND HERDSON, 1973; D'AGOSTINO, 1963; DORIGOTTI ET AL., 1969; FERRANS, BUJA AND MARON, 1975; GABBIANI, BADONNEL AND RONA, 1975; HALL AND SMITH, 1968; HANDFORTH, 1962; HEGGVEIT, 1965; HEGGVEIT AND SADKARNI, 1971; HERDSON, SOMMERS AND JENNINGS, 1965; HIBBS ET AL., 1965; HUTTER, HELLINEK AND KERENYI, 1968; JENNINGS, BAUM AND HERDSON, 1965; LEBRAK ET AL., 1973; LEHR, 1969; METTLER, YOUNG AND WARD, 1977; NOLNER, LARSEN AND SPARGO, 1962; MORIN AND COTE, 1972; NAYLER ET AL., 1968; PRICE, PEASE AND PEARSON, 1962; REICHENBACK AND BENDITT, 1970; RONA ET AL., 1959; RONA, KAHN AND CHAPPEL, 1963; RONA AND CHAPPEL, 1973; ROSENOFF ET AL., 1975; SELYE, 1961; STILL AND SCOTT, 1966; SULKIN AND SULKIN, 1965; SUSIN AND HERDSON, 1967; VAN VLEET, FERRANS, RUTH, 1977a & b; WOOLF ET AL., 1978	PROGRESSIVE PATHOLOGICAL CHANGES WERE IN MOST CASES DOSE-DEPENDENT. CHANGES ADVANCED FROM MILD ALTERATIONS IN THE MITOCHONDRIA AND INTERSTITIAL EDEMA TO DEGENERATED MITOCHONDRIA, DISRUPTED MYOFILAMENTS, NUCLEAR PLEOMORPHISM, MYOCYTOLYSIS AND FIBROSIS. GROSS OBSERVATION IS AN INSENSITIVE ESTIMATION OF CARDIOTOXICITY. LIGHT MICROSCOPIC EXAMINATION CAN REVEAL GENERAL STRUCTURAL CHANGES; HOWEVER, HISTOLOGIC EXAMINATION IS THE MOST SENSITIVE METHOD OF DETECTING MYOCARDIAL DAMAGE. IT ALLOWS THE LOCATION OF ULTRASTRUCTURAL CHANGES WITHIN SPECIFIC AREAS OF THE MYOCARDIUM.

APPENDIX B

MEASUREMENTS OF VASCULAR STRUCTURAL DAMAGE

TABLE B-1

MEASUREMENTS OF VASCULAR STRUCTURAL DAMAGE

TEST SYSTEM PARAMETERS	SPECIFIC TECHNIQUE EMPLOYED	TEST SYSTEM UTILIZED	SUBSTANCES OR CONDITION TESTED	REFERENCES	COMMENTS
LIGHT AND ELECTRON MICROSCOPY	CAPILLARY CHANGES IN THE ANTERIOR WALL OF THE LEFT VENTRICLE	RABBITS	2,000 RADS OF RADIATION	FAJARDO AND STEWART, 1971	MOST INVESTIGATORS EXAMINING THE MICROVASCULATURE FOR DAMAGE HAVE BEEN INTERESTED IN STUDYING CAPILLARY THROMBOSIS AND NARROWING OF LUMEN. THESE TECHNIQUES ARE USED SOLELY FOR RESEARCH PURPOSES AND HAVE LIMITED USEFULNESS IN THE SCREENING OF TOXIC CHEMICAL AGENTS.
	CAPILLARY CHANGES IN THE GASTROINTESTINAL MUCOSA AND KIDNEYS	RABBITS	THROMBIN, HISTAMINE, ε-AMINOCAPROIC ACID, NOREPINEPHRINE, EPINEPHRINE	McKAY, LINDER AND CRUSE, 1971	
	BUCCAL MUCOSAL CAPILLARIES, FEMORAL AND CONTRALATERAL VEIN, MYOCARDIUM, KIDNEYS AND LUNG MICROCIRCULATORY VESSELS, CAROTID ARTERIES	RATS, RABBITS, PIGS	SODIUM WARFARIN, ADENOSINE DIPHOSPHATE (ADP), ADENOSINE MONOPHOSPHATE (AMP), X-RAY, CHOLESTEROL, STASIS THROMBIE, MECHANICAL TRAUMA, THERMAL INJURY, SILK SUTURE, COLLAGEN THREADS	AMROMIN ET AL., 1964; ASHFORD AND FREIMAN, 1967; JØRGENSEN ET AL., 1967, 1970; KAHN, JOHNSON AND DEGRAFF, 1971	
	CREMASTER MUSCLE CAPILLARIES AND VENULES, MICROVASCULATURE IN SKIN FOLD BETWEEN CORACOID AND CARPAL BONES OF THE WING, MICROVASCULATURE IN THE INTESTINE AND MESENTERY	RATS, CHICKENS	THERMAL INJURY, ^{60}CO γ-RADIATION, CARBON VASCULAR LABELING	COTRAN, 1967; STEARNER AND CHRISTIAN, 1971	
VASOCONSTRICTION, VASCULAR RESISTANCE, BIOCHEMICAL DETERMINATIONS	ISOLATED MESENTERIC ARTERY, GRACILIS MUSCLE	DOGS	NOREPINEPHRINE, NORADRENALINE, ISOPROTERENOL, THEOPHYLLINE, VASOPRESSION, ANGIOTENSION II, DIAZOXIDE, SODIUM NITRITE, COCAINE, PHENTOLAMINE	MCNEILL ET AL., 1969; POWELL ET AL., 1971	

APPENDIX C

FUNCTIONAL MEASUREMENTS OF MYOCARDIAL DAMAGE

TABLE C-1

FUNCTIONAL MEASUREMENTS OF MYOCARDIAL DAMAGE

PARAMETER	SPECIFIC MEASUREMENTS	SPECIES OF ANIMAL USED	SUBSTANCES OR CONDITION TESTED	REFERENCES	COMMENTS
FUNCTIONAL EVALUATION	ELECTROCARDIOGRAM (ECG), ARTERIAL BLOOD PRESSURE, LEFT VENTRICULAR PRESSURE, AORTIC FLOW, AORTIC PRESSURE, ARTERIAL BLOOD GAS AND pH VALUES, CORONARY FLOW, LEFT VENTRICULAR END-DIASTOLIC PRESSURE, CALCULATED VALUES: CARDIAC OUTPUT, CARDIAC INDEX, STROKE VOLUME, CARDIAC WORK, MEAN CARDIAC POWER, TOTAL PERIPHERAL RESISTANCE	RATS, RABBITS, DOGS, MONKEYS	CARBON MONOXIDE, CADMIUM ACETATE, ETHANOL, TOLUENE, FLUOROCARBONS, ZYLOSIN, DIAZOXIDE, 1-9- ACETYLMETHADOL, PHENTOBARBITAL, DESOXYCORTICOSTERONE ACETATE, BACILLUS ANTHRACIS METABOLITES, DECREASED AND INCREASED PRELOAD, INCREASED AFTERLOAD, ISOPROTERENOL, PROPRANOLOL, ADENOSINE, REACTIVE HYPEREMIA, CORONARY ARTERY OCCLUSION, ACUTE HEMORRHAGE	ADAMS AND COLE, 1975; BELLAMY, 1978; BOOTH, 1962; BROBMANN ET AL., 1970; CRAMLET, ERICKSON AND COKMAN, 1975; LIU AND WILLIAMS, 1971 ; LIU, 1976, MAHER ET AL., 1977; MORVAI AND UNGVARY, 1979; PASYK ET AL., 1971; PENNEY, SODT AND CUTILLETTA, 1979; REGAN, ET AL., 1969; ROWE ET AL., 1969; ROWE ET AL., 1963; SCOTT AND COWLEY, 1969: STANTON AND WHITE, 1965; TAYLOR AND DREW, 1975; THIND ET AL., 1970; WALSH, TSUCHIYA AND FROHLICH, 1976; ZECH ET AL., 1974	MOST FUNCTIONAL MONITORING TECHNIQUES ARE INVASIVE AND REQUIRE SOME SURGICAL PROCEDURE. THE MOST COMMON INSTRUMENTATION IS IMPLANTED CATHETERS EMPLOYING PRESSURE TRANSDUCERS. MOST OF THE INDIRECT NONINVASIVE TECHNIQUES ARE NOT AS RELIABLE IN LABORATORY ANIMALS AS THE INVASIVE TECHNIQUES. MOST FUNCTIONAL MONITORING TECHNIQUES ARE TOO INVOLVED TO BE USEFUL IN A SCREENING PROGRAM AND LITTLE BASELINE INFORMATION IS AVAILABLE FOR FUNCTIONAL PARAMETERS IN SMALL ANIMALS.
ELECTRICAL CONDUCTION	ELECTROCARDIOGRAM (ECG)	RATS, RABBITS, DOGS	ETHANOL, TOLUENE, ISOPROTERENOL, SALBUTAMOL, HYDRALAZINE, DIAZOXIDE, SKF-A, SKF-B, SKF-24260, CL-A, CANTHARIDIN, PHENTOBARBITAL, PROPOXYPHENE, SEVERE HEMORRHAGE	BALAZS, 1973; BOOTH, 1962; MORVAI AND UNGVARY, 1979; PAGE ET AL., 1979; RABKIN ET AL., 1979	THE ECG'S OF OVER 460 LABORATORY ANIMALS INCLUDING RATS, HAMSTERS, RABBITS, CATS, DOGS, AND PIGS ARE CURRENTLY BEING EVALUATED FOR THE POTENTIAL TO DETECT EARLY MYOCARDIAL DEGENERATIVE CHANGES INDUCED BY TOXIC CHEMICALS (VANARSDEL, 1979). THIS TECHNIQUE MAY PROVE TO BE USEFUL IN A SCREENING PROGRAM.

APPENDIX D

BIOCHEMICAL MEASUREMENTS OF MYOCARDIAL DAMAGE

TABLE D-1

BIOCHEMICAL MEASUREMENTS OF MYOCARDIAL DAMAGE

TEST SYSTEM PARAMETER(S)	QUANTITATIVE MEASUREMENTS	SPECIES OF ANIMALS USED	SUBSTANCE(S) TESTED	REFERENCES	COMMENTS
SERUM AND TISSUE ELECTROLYTES SERUM ENZYMES	Ca, Mg, Na, K CONCENTRATIONS WERE DETERMINED IN SERUM AND TISSUES; VENTRICULAR MYOCARDIUM CPK AND LDH ACTIVITIES IN SERUM	RATS, RABBITS	ADRIAMYCIN	OLSON ET AL., 1974; OLSON AND CAPEN, 1978	THE SERUM ELECTROLYTES WERE NOT SIGNIFICANTLY ELEVATED. MARKED INCREASES IN TISSUE Ca, Na AND H_2O LEVELS AND MARKED DECREASES IN Mg AND K WERE OBSERVED BASED ON WET TISSUE WEIGHTS. ELEVATIONS IN VENTRICULAR TISSUE H_2O WAS CONSISTENT WITH THE MARKED EDEMA AND INTRACELLULAR VACUOLIZATION. THE SERUM ENZYMES LACTIC DEHYDROGENASE (LDH) AND CREATINE PHOSPHOKINASE (CPK) WERE SIGNIFICANTLY INCREASED. MONITORING SERUM ENZYME LEVELS MAY PROVE USEFUL IN A SCREENING PROGRAM FOLLOWING FURTHER DEVELOPMENT IN SMALL ANIMALS. BASELINE VALUES WILL NEED TO BE ESTABLISHED FOR SERUM ENZYME LEVELS. MONITORING TISSUE ELECTROLYTE LEVELS WILL HAVE LIMITED APPLICATION IN A SCREENING PROGRAM.
	Ca VENTRICULAR MYOCARDIUM LEVELS	RATS, GUINEA PIGS	ISOPROTERENOL, 9-a-FLUOROCORTISOL ACETATE, DIHYDROTACHYSTEROL, (NaH_2PO_4), VERAPAMIL D600, PRENYLAMINE	BLOOM AND DAVIS, 1972; FLECKENSTEIN ET AL., 1973, 1974; LEHR ET AL., 1966; LEHR, 1969	ELECTROLYTE SHIFTS COULD BE CORRELATED WITH CYSTIC DISINTEGRATION OF MITOCHONDRIA AND ENLARGEMENT OF THE SARCOTUBULAR SYSTEM. Ca OVERLOAD INITIATES BREAKDOWN OF ATP AND CP.

TABLE D-1 (CONCLUDED)

TEST SYSTEM PARAMETER(S)	QUANTITATIVE MEASUREMENTS	SPECIES OF ANIMALS USED	SUBSTANCE(S) TESTED	REFERENCES	COMMENTS
	Ca, Mg AND Na MYOCARDIAL TISSUE LEVELS	RATS, RABBITS DOGS	ISCHEMIC INJURY	ITO AND CHIDSEY, 1972; LEHR, 1969; LEHR AND CHAU, 1973; SHEN AND JENNINGS, 1972	TRANSIENT ISCHEMIA CAUSED MARKED INCREASES IN Ca LEVELS, HOWEVER, PERMANENT ISCHEMIA SHOWED NO SIGNIFICANT CHANGES. IN BOTH TRANSIENT AND PERMANENT ISCHEMIA THERE WERE MARKED INCREASED IN Na AND TISSUE H_2O LEVELS AND DECREASES IN K AND Mg LEVELS.
PROTEIN SYNTHESIS	^{3}H-LEUCINE; ^{14}C-LEUCINE; THYMIDINE-METHYL-^{3}H; URIDINE-5-^{3}H	MICE, RATS	ADRIAMYCIN, DAUNOMYCIN, HYPERTROPHY	ARENA ET AL., 1974; RABINOWITZ, 1973	MARKED CHANGES WERE OBSERVED IN THE NUCLEIC ACID AND PROTEIN SYNTHESIS IN TREATED HEARTS. STRESS AND WORK-INDUCED HYPERTROPHY INCREASED SYNTHESIS OF NUCLEIC ACIDS AND PROTEINS.

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APPENDIX E

CULTURED HEART CELLS IN MEASURING MYOCARDIAL DAMAGE

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EVALUATION OF SHORT-TERM BIOASSAYS TO PREDICT FUNCTIONAL IMPAIR--ETC(U)
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TABLE E-1

CULTURED HEART CELLS IN MEASURING MYOCARDIAL DAMAGE

TEST SYSTEM PARAMETERS	SPECIFIC TECHNIQUE EMPLOYED	TEST SYSTEM UTILIZED	SUBSTANCES OR CONDITIONS TESTED	REFERENCES	COMMENTS
BEATING ACTIVITY, MEMBRANE POTENTIALS, ACTION POTENTIALS, MORPHOLOGICAL CHANGE, PERCENT VIABILITY, CELL GROWTH INHIBITION, LACTIC DEHYDROGENASE (LDH), LDH ISOENZYMES, CREATINE PHOSPHOKINASE (CPK), ACID PHOSPHATASE, SUCCINIC DEHYDROGENASE (SDH), ENERGY PRODUCTION-ADENOSINE TRIPHOSPHATE (ATP) AND PHOSPHORYL CREATINE, TOTAL PROTEIN, MYOSIN, AMINO ACID INCORPORATION, DNA AND RNA SYNTHESES, CALCIUM EXCHANGE, CYTOCHEMICAL STAINING, FATTY ACID OXIDATION, $^{14}CO_2$ METABOLISM AND PRODUCTION, LYSOSOMAL MEMBRANE PERMEABILITY, LYSOSOMAL FRAGILITY, MITOCHONDRIAL FRAGILITY	PRIMARY CULTURES OF MYOCARDIAL CELLS; MONOLAYERS, CLUSTERS, THIN STRANDS, SPHEROIDAL AGGREGATES	MICE (FETUSES, NEONATAL), RATS (NEONATAL), CHICKENS (EMBRYOS)	ADRIAMYCIN, CARNITINE, OUABAIN, HALOTHANE, TETRODOTOXIN, ACETYLCHOLINE, EPINEPHRINE, NOREPINEPHRINE ATROPINE, D-TUBOCURARINE, PROSTAGLANDINS (PGE_2 AND $PGF_{2\alpha}$), COCAINE, TETRACAINE, DIAZEPAM, BUTYLATED HYDROXYTOLUENE (BHT), BUTYLATED HYDROXYANISOLE (BHA), CHLORPROMAZINE, AMPHOTERICIN B, CLOFIBRATE, HYDROCORTISONE, STEARIC, OLEIC AND LINOLEIC ACIDS, VITAMIN A, CAFFEINE, 2,4-DINITROPHENOL, 2-DEOXYGLUCOSE, BIOLOGICALLY ACTIVE CATIONS (E.G. Li^+, Ba^{2+}, Sr^{2+}, Ca^{2+}) VARIATIONS IN OXYGEN, GLUCOSE, TEMPERATURE, pH, K^+, Na^+, Cl^-, APPLIED CURRENT PULSES	ACOSTA AND WENZEL, 1974, 1975; ACOSTA AND PUCKETT, 1977; ACOSTA AND CHAPPELL, 1977; ACOSTA AND ANUPORO, 1977 - 1978; ACOSTA, PUCKETT AND MCMILLIN, 1978a, b; CHILL, RUMERY AND WOODBURY, 1959; DEHAAN AND FOZZARD, 1975; GOSHIMA, 1975, 1977; KARSTEN ET AL., 1973; LANGER AND FRANK, 1975; LESLIE, GAD AND ACOSTA, 1978; PAPPANO AND SPERELAKIS, 1969a, b; ROSENTHAL, WARSHAW, 1973; SERAYDARIAN, ARTAZA AND GOODMAN, 1977; SPERELAKIS AND LEHMKAHL, 1965, 1968; SPERELAKIS AND SHIGENOBU, 1974; SPERELAKIS AND MCLEAN, 1978; STONG, HARTZELL AND MCCARL, 1975; WENZEL AND ACOSTA, 1973; WENZEL AND ACOSTA, 1976	CULTURED HEART CELLS HAVE MOST OF THE EXPERIMENTAL ADVANTAGES THAT OTHER IN VITRO TESTING SYSTEMS HAVE BESIDES THEIR OWN UNIQUE ELECTRICAL MECHANICAL AND PHYSIOLOGICAL ACTIVITIES. THE CULTURED HEART CELL SYSTEMS NEED FURTHER DEVELOPMENTAL WORK BEFORE THEY WILL BE RELIABLE AND USEFUL IN SCREENING SUBSTANCES FOR CARDIOTOXIC ACTIVITY.

APPENDIX F

TISSUE EXPLANTS AND PERFUSED HEART TECHNIQUES
IN MEASURING MYOCARDIAL DAMAGE

TABLE F-1

TISSUE EXPLANTS IN MEASURING MYOCARDIAL DAMAGE

TEST SYSTEM PARAMETERS	SPECIFIC AREA EMPLOYED	TEST SYSTEM UTILIZED	SUBSTANCES OR CONDITIONS TESTED	REFERENCES	COMMENTS
ACTION POTENTIALS, CONDUCTION VELOCITY, BEATING ACTIVITY, ISOMETRIC TENSION, BIOCHEMICAL AND MORPHOLOGICAL ALTERATIONS	PURKINJI FIBERS, PAPILLARY MUSCLE, LEFT VENTRICULAR APEX, INTERATRIAL AND INTERVENTRICULAR SEPTUM, HIS BUNDLE, SINOARTERIAL AND ATRIOVENTRICULAR NODES	RATS, GUINEA PIGS, RABBITS, CATS, DOGS	QUINIDINE, LIDOCAINE, NOREPINEPHRINE, N-TERT-BUTYLMETHOXAMINE, BRETYLIUM TOSYLATE, ANGIOTENSION II, TEMPERATURE VARIATIONS, REGIONAL REFRACTORINESS, OCCLUSION AND ISCHEMIA	BIGGER AND JAFFE, 1971; CHEN, GETTES AND KATZUNG, 1975; FREEMAN AND TURNER, 1974; FREEMAN, 1977; FREER ET AL., 1976; FRIEDMAN ET AL., 1973; HERDSON, KALTENBACH AND JENNINGS, 1969; LAZZARA ET AL., 1976; WASTILA ET AL., 1972; WIT, STEINER AND DAMATO, 1970	MOST TISSUE PREPARATIONS ARE PERFUSED WITH MEDIUM IN PERFUSION CHAMBERS. EXPLANT TECHNIQUES ARE PARTICULARLY USEFUL IN STUDYING ISOLATED RESPONSES. THE TECHNIQUES ARE NOT SUFFICIENTLY DEVELOPED FOR USE IN SCREENING POTENTIAL CARDIOTOXICITY.
VASOCONSTRICTION	AORTA SEGMENTS	RATS, GUINEA PIGS, RABBITS, CATS	CADMIUM, MANGANESE, BROMOCHLORODIFLUIROMETHANE, DIAZOXIDE, DIBENZOXAZEPINE, PROSTAGLANDINS E_2 AND PGH_2	BULT AND BONTA, 1976; DAVIS AND BACK, 1978; LUNDY, 1978; PORTER, MIYA AND BOUSQUET, 1975; WOHL, HAUSLER AND ROTH, 1968b	AORTA SEGMENTS ARE A USEFUL MODEL IN STUDYING VASOCONSTRICTION

TABLE F-2

PERFUSED HEART TECHNIQUES IN MEASURING MYOCARDIAL DAMAGE

TEST SYSTEM PARAMETERS	SPECIFIC TECHNIQUE EMPLOYED	TEST SYSTEM UTILIZED	SUBSTANCES OR CONDITIONS TESTED	REFERENCES	COMMENTS
BEATING ACTIVITY, CONTRACTION FORCE, ELECTRICAL PHYSIOLOGY, PERFORMANCE CHARACTERISTICS, ETC., [illegible], CORONARY FLOW, MORPHOLOGICAL AND BIOCHEMICAL ALTERATIONS, X-RAY MICROANALYSIS FOR CALCIUM, ZINC AND POTASSIUM, LACTATE DEHYDROGENASE (LDH), CREATINE PHOSPHOKINASE (CPK), GLUCOSE, LACTATE, PHOSPHORYLASE, PHOSPHORYLASE KINASE, ADENOSINE MONOPHOSPHATE (AMP), GUANOSINE, 3',5'-MONOPHOSPHATE (GMP), PROSTAGLANDIN, INHIBITION	PERFUSED HEART	MOUSE (FETUSES) RATS, GUINEA PIGS, RABBITS, CHICKENS (EMBRYOS), CATS, DOGS	CADMIUM, LEAD, CALCIUM, POTASSIUM CHLORIDE, POTASSIUM CYANIDE, SODIUM, CARBON MONOXIDE, EPINEPHRINE, NOREPINEPHRINE, ACETYLCHOLENE, HISTAMINE, ADRENALINE, PROPRANOLOL, 1-ISOPROTERENOL BITARTRATE, METHYLXANTHINES, AMIODARONE, CHLOROPROMAZINES, NITROGLYCERIN, PHENTOLAMINE MESYLATE, DINITROPHENOL, MYRISTIRIC ACID, UREA, NICOTINE, ANGIOTENSIN II, BUTYLATED HYDROXYTOLUENE (BHT), OUABAIN, ENDOTOXIN, ACONITINE, DI-2-ETHYLHEXYL PHTHALATE, DAUNOMYCIN, DIAZOXIDE, OLIGOMYCIN, HYPOXIA, ISHCEMIA	ARONSON, AND SERLICK, 1976, 1977; ARONSON, SERLICK AND PRETI, 1978; BLUKOO-ALLOTEY, VINCENT AND ELLIS, 1969; BRAUNWALD, SARNOFF AND STAINSBY, 1958; CHEUNG AND WILLIAMSON, 1965; DHALLA ET AL., 1978; DIXON, FOZARD AND GOSLING, 1978; DRUMMOND, DUNCAN AND HERTZMAN, 1966; DRUMMOND AND HEMMINGS, 1973; FREER ET AL., 1976; GAD, LESLIE AND ACOSTA, 1979; GARDNER AND ALLEN, 1977; GEORGE ET AL., 1970; HERMAN AND VICK, 1970; HINSHAW ET AL., 1970, 1971, 1972 a&b; HORN, LEVIN AND HAUGAARD, 1969; IMAI ET AL., 1978; JOSEPHSON ET AL., 1976; KALLFELT, WALDENSTROM AND HJALMARSON, 1977; KOPP ET AL., 1978; LAZZARA, EL-SHERIF AND SCHERLAG, 1973; LUBBE ET AL., 1979; MCGRATH, CHEN AND VOSTAL, 1978; NAYLER ET AL., 1968; ROBINSON ET AL., 1965; SCHNEIDER AND SPRELAKIS, 1974, 1975; TAKENAKA AND TAKEO; 1976; TANZ AND OPIE, 1978; TZIVONI ET AL., 1978; VINCENT AND ELLIS, 1963; WENNMALM, 1977, 1978; WIEDMEIER AND SPELL, 1977; WILDENTHAL, 1971; WILLIAMSON, 1964	THE LANGENDORFF PERFUSON TECHNIQUE IS MOST COMMONLY USED. THOUGH, SOME VERY SMALL HEARTS (MOUSE FETUS HEARTS) HAVE BEEN CULTURED IN MEDIA (WILDENTHAL, 1971). A FEW MINOR MODIFICATIONS IN THE LANGENDORFF TECHNIQUE HAVE BEEN MADE TO ACCOMODATE SPECIFIC EXPERIMENTAL PROCEDURES SUCH AS HIS BUNDLE ELECTRODE MONITORING (KOPP ET AL., 1978). ALTHOUGH INVOLVED, THE PERFUSED HEART TECHNIQUES MAY BECOME USEFUL AFTER ADDITIONAL DEVELOPMENT IN SCREENING TOXIC SUBSTANCES.

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